CALLUS PRODUCTION, PLANT REGENERATION AND EVALUATION OF SOMACLONES OF BUFFEL GRASS (*CENCHRUS CILIARIS* L.)

THESIS

SUBMITTED TO

BUNDELKHAND UNIVERSITY, JHANSI

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

BY

ANUPAMA AGARWAL

M.Sc. (Botany)

ICAR

IGFRI

DIVISION OF CROP IMPROVEMENT,

INDIAN GRASSLAND AND FODDER RESEARCH INSTITUTE

JHANSI - 284003

2002

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Affectionately Dedicated to My Late Brother

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Certificate

It is certified that the thesis entitiled "Callus production, plant regeneration and evaluation of somaclones of buffel grass (*Cenchrus ciliaris* L.)" is an original piece of work done by Anupama Agarwal, M.Sc. (Botany) under my supervision and guidance for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi.

I, further certify that:

- It embodies the original work of candidate herself.
- It is up to the required standard both in respect of its contents and literary presentation for being referred to the examiners
- The candidate has worked under me for the required period at Indian Grassland, Fodder and Agroforestry Research Institute, Jhansi (previously known as Indian Grassland and Fodder Research Institute, Jhansi).
- The candidate has registered the required attendance and worked under me for the required period at Indian Grassland Fodder and Agroforestry Research Institute, Jhansi.

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Declaration

I, hereby, declare that the thesis entitled, "Callus production, plant regeneration and evaluation of somaclones of buffel grass (*C.ciliaris* L.)", submitted by me for the award of degree of Doctor of Phylosophy in Botany, in Faculty of Science, Bundelkhand University, Jhansi, is the original piece of work done by me under the supervision of Dr. M.G. Gupta, Principal Scientist, I.G.F.A.R.I., Jhansi and to the best of my knowledge and part or whole of this thesis work has not been submitted for any degree or any other qualification of any university or examining body in India or any university elsewhere.

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December, 18,2002

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List Of Abbreviations Used In The Text And Their Expansion

2,4-D	2,4-Dichlorophenoxy acetic acid
ACP	Acid Phosphatase
ADF	Acic Detergent Fibre
AI	Availability Index
B_5	Gamborg's Medium
BA	6-Benzyle Adenine
CH	Casein Hydrolysate
CP	Crude Protein
CV	Coefficient of Variation
DMY	Dry Matter Yeild
E	Embryogenic
EST	Estarase
GFY	Green Fodder Yeild
G6PDH	Gluco-6-Phosphodehydrogenase
IBA	Indole Butyric Acid
Kin	Kinetin
MS	Murashige and Skoog Medium
NDF	Neutral Detergent Fibre
NE	Non-embryogenic
PGI	Phosphoglucoisomerase
PGM	Phosphoglucomutase
SD	Standard Deviation
SH	Schenk and Hilderbrandt Medium
SOD	Super Oxide Dismutase

INTRODUCTION

1. Introduction

The genus *Cenchrus* belongs to the sub-family Panicoideae and the tribe Paniceae of the grass family Gramineae (Poaceae). It comprises of an ubiquitous group of grasses inhabiting wastelands, deranged or staggered areas and sandy soils in the warmer regions of both the hemispheres. Because of spiny nature of their inflorescence, members of this genus have been avoided by the human co-inhabitants. However, a few species with less spiny inflorescence and more luxuriant foliage, have been introduced as forage grasses in the areas, especially, in Africa, India and South America (De Lisle, 1963).

1.1 History and Nomenclature

A detailed discussion of the pre-Linnaean names applied to this genus is found in chase (1920). Linnacus described this genus in 1742 and the name "*Cenchrus*", had been derived from the Greek word "Kenchorus", which referred to some from the millet (Gunther, 1934). According to the International Rules (Lanjouw, 1961), the valid name (*Cenchrus*) dates from Linnaeus Species Planterum (1753).

Linnaeus (1753) described five species in the genus *Cenchrus*. Poiret (1804) discussed eighteen species in his work on Gramineae, followed by Persoon (1805), who described eleven species. Kunth (1833), in his "Agrostographia", listed forty-five species of *Cenchrus*. Rocmer and Schultes (1817) described twenty-one species of *Cenchrus* in their systema Vegetabilium. Steudel (1855) listed and described thirty species of *Cenchrus*. De Lisle (1963), in his monograph on taxanomy and distribution of *Cenchrus*, has given the key of identification of twenty species of *Cenchrus*. Watson and Dallwitz (1922) in their treatise, "The grass genera of the world", have mentioned twenty-two species of *Cenchrus*.

1.2 Morphology

Cenchrus is a genus of Panicoid grasses with terete and solid clums and fibrous roots. It comprises of both the annual and perennial plants distributed among various species. Annuals are usually solitary or may occasionally from large clumps, whereas the perennials, some of which have bulbous bases, may produce large tussocks or mats. The solid stems are characteristics of the grasses, which are

apparently best able to survive under semi-arid conditions. The leaves are flat, narrow and sometimes folded or involute. The leaf blades are linear or linear lanceolate. The ligule is a fringed membrane or may be reduced to fringe.

1.3 Floral biology

The inflorescence of the members of genus *Cenchrus* is a spike-like panicle consisting of a few to numerous fasciles (Burs or involucre) within which one or more spikelets are enclosed. Sohns (1955) found that the spikelets of *Cenchrus* are terminal in the fascicles and that the spines represent sterile first-order axes whose branches have become fused laterally. The fused branches are the part of an elongated inflorescence whose axis has become shortened and the branches have become sterile. The involuere (bur) of many species in *Cenchrus* represents a high degree of specialization expressed as coalescence of inflorescence branches. The most highly specialized members of this genus appear to have almost complete fusion of the branches into a compact bur and the spines tend to be quite wide and generally united for a considerable distance above the base of the bur. The characteristics are used in the present day treatment of the genus *Cenchrus*. The spikelets consist of a first and second glume, sterile lemma and palea and a fertile floret. The so-called "sterile-lemma" lacks an ovary but usually produces functional stamens with variable pollen. The anthers produced by sterile lemmas are twice the length of those borne in the adjoining fertile florets. The species of the genus *Cenchrus* are markedly protogynous. Emergence of stigma proceeds in basipetal succession.

Self-fertilization is apparently common in most species of *Cenchrus*, but cross-fertilization also occurs normally. The absence of lodicules has been reported in the florets of *Cenchrus* by Arber (1934) and Bor (1960). Apmixis and pseudogamy are reported to be prevalent in *C.cilliaris* and *C.setigerus* (Fisher, Bashaw and Holt, 1954). Seeds of all species of *Cenchrus* require a dormancy of about five to six months.

1.4 Cytology

There are evidently two major groups in the genus Cenchrus with respect to the basic chromosome number. In the group of species largely confined to the western hemisphere, the basic chromosome number appears to be x = 17, while those plants largely confined to Africa and Asia have a basic number x = 9 or 10. Two exceptions of this old world group include *C.prieurii* and *C.biflorus* reputedly having a basic number x = 17.

The second group of species, having basic number x = 9 or 10, includes *C.ciliaris, C. setigerus* and *C.myosuroides*. The presence of a basic number x = 9 in at least the first two species of Cenchrus from an ancestral form with this basic number (De Lisle, 1963).

1.5 Phylogeny

Based on a number of morphological characters for phylogenic considerations, the species of *Cenchrus* fall into two basic groups. The first, presumably more specialized group and homogenous assemblage of species include *C.pilosus, brownii, echinatus, longispinus, tribuloids, insurtus* and *platyacanthus* etc., having basic chromosome number x=17. These are native to the western hemisphere. The second basic group represents heterogenous assemblage of species, consisting of *C.ciliaris, setigerus, multiflorus, perieurii* and *biflorus* etc having basic chromosome numbers x=9. 10. These are restricted to Africa, India and Southern-East Asia. Many of these forms have apparent closer attinities with the members of the genus *Pennisetum* than to their relatives in tropical America.

One suggestion, that the origin of *Cenchrus* in tropical America, has been put forth by Hartley (1950) by summarizing that the Paniceae along among grass tribes has predominantly a New-World distribution, being exceptionally abundant in a well defined region from the Bahmas to south-eastern Brazil. However, there seems little doubt that the members of both *Cenchrus* and *Pennisetum* originated from common ancestral stock. The origin of *Cenchrus* in Afro-Asian regions is suggested by the preporderance of primitive species in those areas. Several species in Africa and India have apparent lower basic chromosome number (x=9,10) and

perennials. According to Stebbins (1950), perennial habit of growth is erally thought to be more primitive, with annual habit a derived condition in ny groups of plants. Camp (1947) suggested the possibility of southern nisphere origin for many angiosperms and the presence of a larger, more ntinuous landmass in the south than now exists. Under such conditions, the cestors of *Cenchrus* and *Pennisetums* could have been quite wide spread with veral centers of dispersal rather than one as proposed by Hartley (1950).

a) *Cenchrus ciliaris*

'enchrus ciliaris L. syn. *Pennisetum cenchroides* Rich; *P.ciliare* (L.) Link, commonly known as buffel grass (Australia), African foxtail (U.S. and Kenya), dhaman grass and anjan grass (India) is a native of tropical and sub-tropical Africa, India and Indonesia (Plate # 1, Fig.1). It is widely distributed in hotter and drier parts of India, Mediterranean region, and tropical and Southern Africa. It is found in open bush and grasslands in its natural habitat. In India it is widely distributed in the plains of Rajasthan, Punjab and Western U.P. extending upto the foothills of Jammu upto 400 m altitude. It is one of the predominant grass species of *Dicanthium-Cenchrus-Lesiurus* grass cover of India (Dabadghao and Shankarnarayan, 1973). It is polymorphic, perinneal and warm season bunch grass with extensive native range. It is highly drought tolerant and well adapted to arid and semi-arid areas and thrives well in light textured soils of high phosphorous status within and optimum range of pH of 7.0 to 8.0. It grows upto a height of 120 cm. It serves as an excellent source of roughage for grazing livestock in western, central and penninsular states of India (Reddy *et al.,* 1955a). Its yield could be increased from 4.8 t/ha to 9.08 t/ha with an application of 168 kg N/ha. Buffel grass makes a reasonable quality of hay when cut in the early flowering stage yielding upto 2500 kf/ha per cut with crude protein content of 6-10% of DM. The old grass after seed harvest gives low quality roughage having crude protein content of 4-6%. It has high oxalate content of 1.2 to 2.8% of total oxalate and has been reported to cause bug-head disease in horses when fed exclusively (Skerman and Riveros, 1990). Its main attributes are its hardness, deep rooting ability to

grow in harsh climate and generally free seeding habit (Plate # 1, Fig.2,3), its persistence and drought tolerance.

C. ciliaris is a highly heterozygous and naturally occurring polyploid species exhibiting vast range of genetic variability in the form of several eco-and biotypes co-existing in the same stand. It is represented by various chromosomal races, such as tetraploids, pentaploids and hexaploids with occasional occurrence of several aneuploids ranging from 2n=36 to 2n=54, having basic chromosome number x=9. The tetraploids are the most common representatives of species and are obligate apomicts in their mode of reproduction, though sexually reproducing plants also occur occasionally in nature.

(b) Cenchrus setigerus

Cenchrus setigerus Vahl.(syn. *C.setiger* Vahl.), commonly known as birdwood grass (Australia) and modadhaman grass (India), is a native of Africa from the Nile valley to the Red-sea and eastward through Arabia to India. It is an adventive to the southern United States, Australia and South America where it has been introduced as the experimental forage grass. It is well adapted to arid and semi-arid conditions better than *C.cilliaris*. In its natural habitat, it is also found in open bush and grasslands and thrives well on alkaline soil. It is highly palatable and nutritious perennial forage grass suitable for both grazing as well as stocking purpose. Fresh material at early-bloom stage contains 17.0 to 18.6% crude protein content (Skerman and Riveros, 1990). It is extremely tolerant to heat and drought and grows well in areas of rainfall as low as 200 mm which makes it excellent for improvement of low rainfall grazing lands. Because of its hardy nature and ability to grow in low rainfall areas, it serves as a valuable stand-over feed in these areas. It also prefers light textured soil but does well over a wide range of soils than *C.ciliaris*.

C.setigerus is a tufted perennial grass with flat or folded leaf blades and grows upto 60 cm in height (Plate # 1, Fig.1). It is somewhat similar to the buffel grass in appearance but the seeds of grass carry short spine-like bristles and are less fluffy (Plate # 1, Fig.3). It has dense false-spike 1.5-1.9 cm long and spikelets, 3.0-4.5 mm long surrounded by a rigid involucre 3.5-5.0 mm length (Plate # 1, Fig.2). The

outer bristles are minor or absent having an inner flattened grooves on the back. Each cluster of spikelet contains 1-3 caryopsis. The inner bristles in contrast to *C.ciliaris* are glabrous, connate for 1-3 mm from the base, flat and rigid throughout.

C.setigerus is also an apomictic grass in its reproductive behavior but pollination is necessary for endosperm formation and seed set. Presence of apomixis in *C.setigerus* was reported by Fisher *et al.* (1954). Synder *et al.* (1955) observed one to four embryo sacs per ovule in this species. Aposporous formation of embryo sac in *C.setigerus* takes place, in a manner similar to that of *C.ciliaris*, from enlarged nuclear cells following megasporogenesis upto four-nucleate stage. It is also a tetraploid species having chromosome number 2n=36 with a basic chromosome number of x=9 as has been reported by Darlington and Wylie (1955).

(c) *Cenchrus echinatus*

C.echinatus L, commonly known as "hedgehog grass", is an annual grass of the tribe Paniceae. It is widely distributed through out the warmer regions of southern United States, Central and South America and the West Indies. It is a native of the new world and is adventive and widely distributed in most of the Pacific Islands, the Philippines and Australia probably as a result of man's activities. The species has also been introduced into Africa and has spread over the coastal pain of Israel. In India, it is found in the plains of north west region and grows well on sandy soils.

C.echinatus has trete clums, 15-85 cm tall, leaf sheath compressed, ligule ciliate, leaves galbrous to pubescent, inflorescence open 2.0-10.0 cm long (Plate # 1, Fig.1). The burs are large and widely spaced on the inflorescence (Plate # 1, Fig.2). The spine tips in mature burs tend to assume a purple tinge (Plate # 1, Fig.3). Pubescence is highly variable in *C. echinatus*. Some plants are completely glabrous while others may possess rather densely villous sheaths and blades. A chromosome number of 2n=34 has been reported in *C. echinatus* by Avdulove (1931). However, Nuenz (1952) and De Lisle (1963) reported a somatic number 2n=68. It appears that *C.echinatus* L. is probably a tetraploid form derived from some ancestor with a basic chromosome number x-17. Gupta *et al.* (2001) have reported this species as

Plate # 1

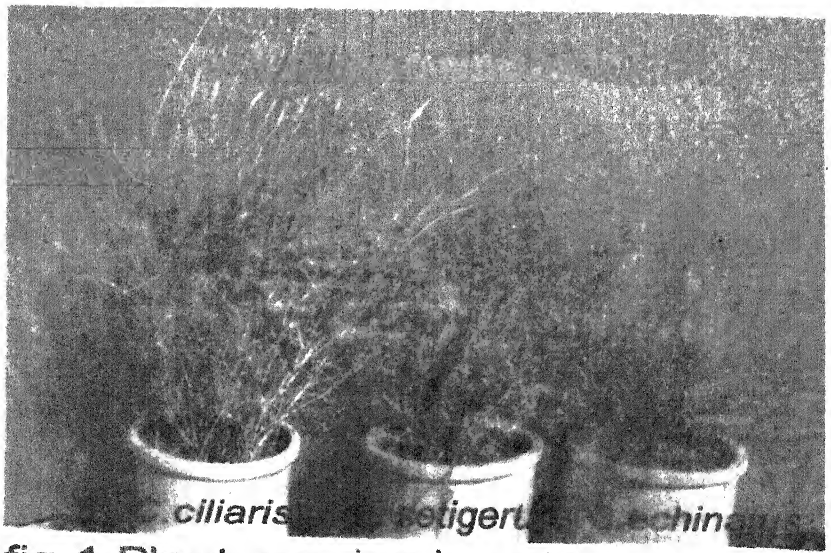


fig-1 Plant growing in pots

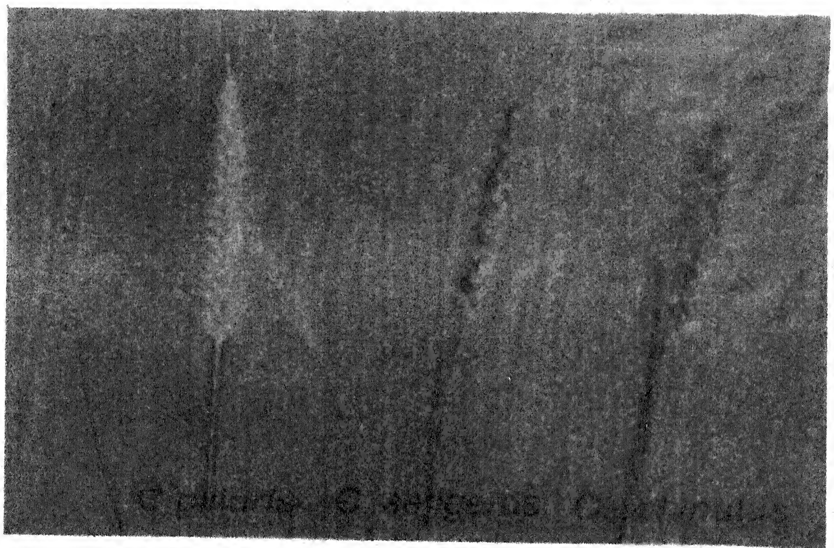


fig-2 Immature Inflorescence

fig-3 Seeds

an obligate sexual in its reproductive behavior. It is much valued as a forage grass on account of early appearance of its foliage.

1.6 Biotechnology

Regeneration of plants from cell and tissue culture is one of the most important and essential component of biotechnology that is required for the genetic manipulation and improvement of plants (Vasil and Vasil, 1986). A large number of grass species and cereals which are most important sources of fodder and food were generally considered to be rather recalcitrant to plant tissue culture responses two decades ago. Though, regeneration of plants of many grass species through tissue cultures were reported, the regenerative ability was transient, sporadic and limited to a few genotypes only (Vasil, 1983). There has been a tremendous improvement in the ability to obtain long term and high frequency regeneration from tissue cultures of the graminaceous species since 1980s (Vasil and Vasil, 1986) and as a consequence, the *in vitro* regenerated plants, i.e., somaclones in several grass and cereal crops, have been produced and evaluated for their beneficial variation. There are convincing reports of occurrence of somaclonal variation among plants regenerated through tissue culture (Larkin and Skowcroft, 1981 and Lorz *et al.*, 1988).

In the crops like sugarcane and potato, somaclonal variation heralded a promising future of combating with pests and quality problem (Heinz *et al.*, 1977). Single gene mutation and organelle mutations have been produced by somaclonal variation. By introducing the best varieties into cell culture and selection for improvement of a specific character, the somaclonal variation could be used to uncover novel character of a new variety that remain all the beneficial traits of an existing variety while adding some additional desirable traits.

Being apomictic, the genetic improvement of *C.ciliaris* and *C.setigerus* through conventional breeding methods is very difficult and is restricted to exploiting natural variation only. The undesirable gene combinations in such selections are unavoidable and interfere in rapid progress of genetic improvement. Somaclonal variation could be greatly beneficial which can supplement and complement the

conventional breeding efforts in Cenchrus species. For generating somaclonal variation, efficient tissue culture protocol systems for callus induction, maintenance of calli and regeneration of *in vitro* plants are the most important prerequisites. Such optimized protocols for *in vitro* plant regeneration procedures in *Cenchrus* species have not been available and culture responses differ from genotype to genotype. Keeping in view the above back – ground, the present study was conducted with the following objectives:

1. To optimize the *in vitro* conditions for callus formation and regeneration of plants from different explants of different genotypes of *Cenchrus* species.
2. To investigate the potential of somaclonal variation induced *in vitro*.
3. To study the stability of somaclonal variation by evaluating the performance of the progenies of somaclones.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Plant cell and tissue culture

The regeneration of plants from cell & tissue culture is an important and essential component of biotechnology that is required for the genetic manipulation and improvement of plants. Cell and tissue culture technology is increasingly being applied for genetic improvement of a wide range of crop plants. The inception of plant tissue culture dates back after a German botanist **Haberlandt** (1902) who postulated the concept of totipotency in plant cells followed by the work of **White** and **Gautheret** in 1934. They independently established tissue cultures in some plant species. Tissue culture technology may provide rich and novel source of variability with a great potential for crop improvement without resorting to mutation or hybridization. A high rate of regeneration from callus culture is a prerequisite for the use of tissue culture as a tool in the crop improvement. Cereal and other grass species were generally considered to be rather recalcitrant to plant tissue culture technique until early 1960s. In the late 1960s, the reports started coming on regenerable cultures from monocots on simple media supplemented with 2,4-D. the first convincing description of plant regeneration in monocots is from tissue culture of rice (Tamura, 1968) and sugarcane (Heinz and Mee, 1969).

2.2 Cell and tissue culture in Gramineae

Callus culture response occurs throughout the plant kingdom (Goutherete, 1959; White, 1963 and Butenko, 1964). In beginning, the majority of reports in the literature were from investigations on dicotyledonous plants and the account of callus formation from monocotyledons were not as numerous (Straus, 1954; Norstog, 1956; Tamaoki and Ullstrup; 1958 and Carter *et al.*, 1967).

Though regeneration of plants from tissue cultures of many grass species were reported, the regenerative ability was transient, sporadic and limited to a few genotypes only (Vasil, 1983). 1980 onwards, there has been a tremendous improvement in the ability to obtain long term and high frequency regeneration from tissue culture of the graminaceous species. A brief account of various factors

determining the success of plant tissue culture and their optimisation in Gramineae is described here, both for callus formation and morphogenesis.

2.2.1 Callus induction

When the cells from differentiated tissues are grown on artificial nutrient medium with certain hormones, the mature cells revert to the meristematic state and form undifferentiated mass of cells. This mass of cells is termed as callus and phenomenon is called dedifferentiation. Totipotency is probably a characteristic of all living plant cells and it may be consistently expressed by all cultivars of a species (Bingham *et al.*, 1975 and Green and Phillip, 1975) or all species of a genus (Saunders and Bingham, 1972). The regeneration of entire plant from the isolated cells affirmed the totipotency of plant cells (Vasil, 1980).

Callus induction occurs in the presence of auxin (Skoog and Miller, 1957). The quality and quantity of callus is affected by many factors.

2.2.1.1 Factors affecting callus formation

(a) Explant

Reproducible regeneration of plants is now possible from tissue cultures of all major cereal and grass species (Vasil, 1983). This is due in part to the realization that explants from mature tissues of the Gramineae generally yield either non- regenerable or only root forming calli. This led to wide use of immature meristematic tissues as explants for the establishment of totipotent cultures.

Immature embryos, young inflorescences and young leaves have proved to be most suitable explants for regeneration. Regeneration is said to occur either by *de novo* formation of shoot primordia (Rangan, 1974 and Nakano and Maeda, 1979) and proliferation of presumptive shoot primordia (King *et al.*, 1978) or somatic embryogenesis (Vasil, 1982 and 1983).

Immature embryos have been the most widely used as explant in many cereals and grasses. This includes rice (Ozava and Komamine, 1989; Bong *et al.*, 1996 and Bozorgipour and Snape, 1997), wheat (Ozias-Akins and Vasil, 1983a;

Larkin *et al.*, 1984; Chawla, 1989; Ito and Abe, 1990; Chauhan and Singh, 1995 and Machi *et al.*, 1998), sorghum (Gamborg *et al.*, 1977; Ma *et al.*, 1987 and Guo and Liang, 1993), oats (Heyser and Nabors, 1982 and Bregitzer *et al.*, 1989), maize (Lowe *et al.*, 1985; Hodges *et al.*, 1986; Vasil and Vasil, 1986; Willman *et al.*, 1989; Garcia *et al.*, 1991 and Madan *et. al.*, 1994), rye (Goldstein and Kronstad, 1986; Taniguchi *et al.*, 1991 and Shakib, 1992) and *Panicum maximum* (Akashi and Adachi, 1991).

Mature seeds, seedlings or their parts and embryos have also been successfully used in rice (Nakano and Maeda, 1979; Heyser *et al.*, 1983 and Abe and Futsuhara, 1986), wheat (Mackinnon *et al.*, 1987), sorghum (Bhaskaran *et al.*, 1985; Cai *et al.*, 1987; Elkonin *et al.*, 1989 and Murty *et al.*, 1990), oats (Carter *et al.*, 1967; Cure and Mott, 1978 and Heyser and Nabors, 1982), maize (Santos *et al.*, 1984; Ochesenu *et al.*, 1990 and Furini and Jewell, 1994), rye (Eapen and Rao, 1982), barley (Jeleska *et al.*, 1984; Rengel and Jeleska, 1986 and Pan and Liange, 1991), finger millet (Eapen and George, 1989) and pearl millet (Nagaratna *et al.*, 1991).

Leaf sheath was used as explant in rice (Bhattacharya and Sen, 1980 and Abdullah *et al.*, 1986), wheat (Zamora and Scott, 1983); sorghum (Wernick and Brettel, 1982 and Wernick *et al.*, 1982), rye (Linacero and Vazquez, 1986) and *Pennisetum* species (Haydu and Vasil, 1981).

Shoot meristem and basal segments of young leaves (Zamora and Scott, 1983) or shoot segment enclosing meristem (Wernick and Milkovits, 1986) were used in wheat. Young leaf tissue did produce regenerable callus in sorghum (Wernicke and Brettell, 1982), rice (Ozava and Komamine, 1989; Bong *et al.*, 1996 and Bozorgipour and Snape, 1997), wheat (Sears and Deckard, 1982 and Linacero and Vazquez, 1986) and *Pennisitum purpurium* (Haydu and Vasil, 1981). Embryogenic callus was also obtained from shoot apex of sorghum (Bhaskaran and Smith, 1988). In barley shoot apex (Cheng and Smith, 1975 and Weigel and Hughes, 1985) and leaf base (Becher *et al.*, 1992) were used for callus production.

Cheema *et al.* (1992) tried axiliary buds and spindle tissues from one-month-old tillers of sugarcane. In maize immature male and female inflorescences,

immature and mature embryos, stem apices, stem and leaf segment were tried by Ochesenu *et al.* (1990). The response to callus culture was dependant on organ explants. Callus formation percentage was low from leaf parts and the callus had poor viability where as callus formation from immature inflorescences and embryos and from stem apices exceeded upto 50% with good proliferation.

Immature inflorescences were good source of regenerable callus in sorghum (George and Eapen, 1988; Murty *et al.*, 1990, and Kosting *et al.*, 1996), barley (Chu *et al.*, 1984), *Pennisetum* species (Vasil and Vasil, 1981; Boiti and Vasil, 1984 and Nagarathna *et al.*, 1991), *Cenchrus* (Sankhla and Sankhla, 1989; Kacher and Shekhawat, 1991 and Murty *et al.*, 1992), sugarcane (Liu, 1993), rice (Li *et al.*, 1990), maize (Rao *et al.*, 1990) and wheat (Zheng *et al.*, 1989).

The other explants used were root tips in rice (Abe and Futsuhara, 1985 and Zimny and Lorz, 1986). Kucherenko and Vlasov (1988) produced callus from individual parts of the embryo of rice. All the parts of embryo were proved capable of forming callus, but only the calli obtained from the scutellum and plumule showed regeneration ability which decreased with increasing subcultures.

Visarda *et al* (1998) established rachilla (the portion joining sterile glume to palea and lemma) as an explant source for callus induction and that was found to be an additional source of embryogenic callus in rice.

Kosting *et al.*, (1996) showed that in sorghum, the immature embryos proved promising but the best results were obtained with young inflorescences. Callus from inflorescence explant (0.2-1.0 cm long) showed the highest frequency of embryoid formation. Kasperbauer (1990) found that successful regeneration depended on explant and tissue source. Murty *et al.* (1990) were able to demonstrate that in sorghum, among the various explants, such as, scutellum from mature seeds, shoot portion of young seedlings and immature inflorescence, the highest regeneration frequency obtained was from immature inflorescence.

Kalmani and Ramasamy (1998) suggested that in pearlmillet, the maximum response for callus induction and regeneration was obtained from inflorescence

followed by leaf explant. Gosal *et al.* (1993) found that in bagar grass, the length of inflorescence was critical for callus induction, the optimal size being 1.5 to 2.5 cm.

Chauhan and Singh (1995) have shown that variability for different plant morphological traits was greatest among regenerants derived from coleoptile nodes as compared to those from mature and immature embryos. Immature embryos collected from the same inflorescence behaved differently in culture medium, depending on their size and location on the inflorescence. Response of explants from well nourished plants were different from those of nutrient deficient plants (Duncan amd Widholm, 1988). The scutellum of immature embryos provided the highest frequency of embryogenic callus production.

Barro *et al.*, (1999) explained that embryogenic capacity in inflorescences was higher than the immature scutella. However, shoot formation from immature scutella was clearly higher than from the inflorescences.

Kalamani and Ramasamy (1998) observed in pearl millet that maximum callus induction and regeneration was obtained from inflorescence followed by leaf expalnts. Frequency of embryogenic calli was highest using immature embryos.

Age of explants

The ontogenic age of the plant, organ or tissue forming as source of explants that are in juvenile phase of development, are more amenable for embryogenic callus induction than those from adult material (Tissarat *et al.*, 1979). Botti and Vasil (1984) obtained embryogenic callus from the shoot meristem, excised along with the two youngest leaf primordia in *Pennisetum americanum,* suggesting that the mature tissue of the explant may have an inhibitory effect on the capacity of the meristematic tissue to form embryogenic callus.

Liu (1993) demonstrated the effect of age of explant in sugarcane by initiating callus from all parts of the infloresence. In terms of callusing frequency, the most important factor was the age of explant. As long as it was in the stages of pollen mother cell to tetard, all the used genotypes were capable to produce large quantity of callus.

Rao *et al.*, (1992) found in sorghum that immature seeds produced more organogenic callus compared to mature seeds. Mature seed-derived callus failed to differentiate while immature seed-derived callus produced good number of plantlets. Calli produced by mature embryos did not have moprphogenic potential (Ozias-Akins and Vasil, 1983b). Sharma *et al.* (1999) observed that 12 days old embryos of a dwarf cultivar and 18 days old embryos of Basmati were not responsive in case of rice.

Zhao-Cheng *et al.* (1999) noticed that mature embryos performed poorly than young panicle in rice for production of regenerating callus. Hui *et al.* (2000) found that stamens and pistil differentiation stages of young panicles gave the best results as explants in rice.

(b) **Genotype**

The most common feature in tissue culture of gramineae is the genotypic variation in culture responses (Bhaskaran and Smith, 1990). Conditions optimum for plant regeneration of one cultivar may not induce regeneration in another cultivar of the same species. This has been a nagging problem in cereal tissue culture and may have contributed to the abundant literature on cereal tissue culture as the investigators had to optimize conditions for individual cultivar. Thus the choice of genotype for *in vitro* studies has a major influence on the probability of successful callus initiation and plant regeneration. Reports have indicated that regeneration from callus cultures is genetically controlled by nuclear genes in maize (Tomes and Smith, 1985 and Hodges *et al.*, 1986). Peng and Hodges (1989) also presented that regeneration ability in rice is under control of both nuclear and cytoplasmic genes. In sorghum, Ma *et al.* (1987) found that the ability to form regenerable callus varied among genotypes which was heritable and acted as a dominant trait. They proposed at least two gene pairs were involved. However, Close and Ludman (1989) presented evidence that the induction of regenerable callus in maize is largely a physiological phenomenon that can be manipulated by the type and concentration of plant growth regulators, independent of the genetic background of the explant.

Some times, environment induced differences may be very difficult to distinguish from true genotypic differences, except under very controlled

conditions. Response of explants from well-nourished plants was different from those of nutrient-deficient plants (Duncan *et al.*, 1985). Cultures from plants grown in summer were known to give response different from plants grown in the cool season (Rines and McCoy, 1981; Hanzel *et al.*, 1985 and Ma *et al.*, 1987). This indicated that something within an explant is as critical for a given response as its genotype. Many of the genetic differences could be circumvented by growing the source plants under optimal conditions and also by varying nutrients and hormones in culture media (Duncan *et al.*, 1985).

In barley, Bregitzer (1992) found that relative growth rate of embryogenic calli and regenerability were influenced by the genotype. Genotype was the most important determinant for the *in vitro* response and the friability and morphology of callus. Callus formation was affected by genotype, medium and their interaction and these effects were statistically significant. Vigorously growing callus cultures tended to be more friable within a particular genotype and not in all genotypes.

Dorosieve *et al.* (1991) observed that in barley, diploid varieties had higher *in vitro* regenerative ability than their tetraploid version and there was a marked association between regenerative ability and the genotype. Ruiz *et al.* (1992) and Dehleen (1999) showed significant effect of genotype on culture in barley.

In wheat, Mathias *et al.* (1988) and El Waffa (1999) observed that proliferation of callus varied among the genotypes. Ahmed (1999) while working on salt stress in callus, found that the genotype were variable with respect to callus when treated with different concentrations of sodium chloride. The genetic variance was extremely higher in magnitude in relation to the environmental variance, when a large number of calli with shoot formation exhibited high values of phenotypic and genotypic coefficient of variation. El Waffa and Ismail (1999) used immature embryo of 16 genotypes of wheat as explant for culture where highly significant differences were observed due to genotype form callus induction, fresh weight/callus number of green spotted calli and number of plantlet regenerated. Hess and Carman (1998) also proved the genotypic influences in wheat.

In rice Gupta *et al.* (1989) and Gao-Zhen *et al.* (1999) explained that callus formation rate and plantlet formation rate were markedly different between genotypes and the genotype was the most important factor influencing plantlet regeneration.

Maheshwari and Rangasami (1989) studied five genotypes of *Oryza* species. The culture requirement differed with cultivars within the species and between the species of the genus. Bai *et al.* (1999) noted in ovary culture of rice that callus induction frequency was dependant on genotype because some genotypes of Indian rice did not respond to callus induction in any of the media.

According to Sat'ya and Kuruegenko (1988) and Ochesanu *et al.* (1990) genetic differences were detected in regenerative ability in maize. The hybrids differed in this respect and had better regeneration ability than inbred lines. To prove the genotype influences, Ting and Schneider (1990) worked with five maize inbreds, which showed abundant proliferation and embryogenesis with some embryogenic areas exhibiting shoot differentiation. One line showed creamy surfaced morphology and adequate proliferation but no embryogenesis, suggesting that response to culture was determined by genotype.

In sugarcane, callus induction was maximum only in some genotypes (Cheema *et al.*, 1992 and Sheng *et al.*,1998). Various types of calli were obtained by Akashi and Adachi (1991) in guinea grass and embryogenic responses were found to be genotype dependent. Cai and Butler (1990), Guo and Liang (1993) and Bhat and Kuruvianashtti (1994) found same results in sorghum. Nagarathna *et al.* (1991) used pearl millet for showing the genotypic effect. Rines and McCoy (1981) and Bregitzer *et al.* (1989) found that genotypes influenced callus initiation frequency and culture quality in oats.

The genotypic differences were mostly due to endogenous hormonal differences (Fitch and Moore, 1990). Even different explants from a single genotype did not respond identically in culture, most likely due to varying gradient of endogenous hormones (Wernick and Bretell, 1982). Thus, the genetic basis of variation in tissue culture response and morphogenesis is most likely due to differences in hormone metabolism within the explant which is

established by the level of gene expression for individual hormones by the genotypes (Norstog, 1970).

(c) Media and Adjuvants

The composition of the culture medium is an important factor in the successful establishment of tissue culture. The basic nutritional requirement of plant cell cultures are very similar to those normally utilized by whole plants. However, the nutrient media which are successfully used for cells, tissue and organs were devised to meet the specific requirements (Murashige and Skoog, 1962 and Schenk and Hildebrandt, 1972). Major elements present in the media has significant role. Chu Chin-Chin (1981) reported that ammonium ions at high concentration apparently inhibited the cell growth *in vitro*. When potassium nitrate or ammonium sulphate were used individually as the sole source of nitrogen, they were not as effective as the two sources used in combination.

In barley, Mordhorst and Lorz (1993) demonstrated that the different components of the media had no effect on the frequency of initial divisions but had significant effect on embryogenesis and plant regeneration. Pan and Liang (1991) reported that callus induction frequency varied with cultivars and the basal medium. The level of potassium di-hydrogen phosphate in the media had a marked effect on the levels of mineral elements in callus.

Callus formation was affected by the genotype, medium and their interaction. These effects were statistically significant (Bregitzer, 1992). Culturing immature embryos of barley on MS medium, as opposed to B_5 and CC medium, consistently produced an equivalent or greater proportion of friable, rapidly growing callus cultures and the relative friability of these cultures was often greatest. B_5 medium was the least efficient and tended to have more necrosis than callus grown on MS and CC medium.

Hassawi *et al.* (1990) and ElWaffa (1999) showed highly significant difference between genotype and medium by analysis of variance. Ito and Abe (1990) tried MS, B_5 and N_6 medium for callus induction in 10 days old embryos of wheat. MS media was found 6-8 times better than the other two media.

Bohorova *et al.* (1995) proved that N6 basal media was better than other media for callus initiation and maintenance in callus culture of maize. When this

media was supplemented with silver nitrate, an increase in the embryogenic callus formation and regeneration potential was observed.

According to Koetji *et al.* (1989) callus growth and plant regeneration were influenced by organic supplements to the basal medium and media solidifying agents in rice.

Studies on sugarcane callus revealed that shoot buds were induced by transferring the callus to modified MS media without growth regulators (Lal and Singh, 1991).

Kalamany and Ramasamy (1998) observed in pearl millet that suitability of the media used for the callus induction was in the order of N_6>MS>White>LS. Lambe *et al.* (1999) found that frequency of friable embryogenic callus is related to the composition of the medium. Same results were observed in tall fescue by Kasperbauer (1990).

The most commonly used carbohydrate for plant tissue culture is sucrose. In nature, carbohydrate is transported within the plants as sucrose and the tissue may have the inherent capacity for uptake, transport and utilisation of sucrose (Eapen and George, 1990). Kavikishor *et al.* (1992) revealed in a study of kodomillet, finger millet and foxtail millet that sucrose had a profound influence on shoot organogenesis in all three millets. According to Jain (1997), beside genetic factors, nongenetic factors such as nature of carbohydrate source in regeneration medium has shown to greatly influence the expression of totipotency. Maltose has been found to be the preferential carbon source for the proliferation of embrogenic callus and shoot regeneration in rice. Sucrose concentration of six per cent was effective as noted by Zhang (1991). Increased frequency was found with eight per cent sucrose in wheat by Mathias *et al.* (1988).

Several media that have been developed for growth of isolated plant cells, tissues and organs show a wide diversity in the concentration of the nutrient components. Thiamine, nicotinic acid, pyridoxine and myo-inositol all appeared to be somewhat stimulatory to atleast some of the species. Starting with the initial medium, higher levels of the major inorganic compounds were found to improve growth. Most effective growth media contain iodine in the

form of potassium iodide. Copper ion appeared stimulatory at a level higher than initially expected. Chelated iron of low level was adequate to maintain the growth in cultures. Maganese, boron zinc and molybednum all appeared to stimulate growth (Roy and Hildebrandt, 1972).

Media adjuvants used to complement with unspecified nutrients/growth factors are often of much help in callus induction. Nevertheless, the use of organic adjuvants like yeast extract, coconut water and their mode of action could not be determined as they are highly variable across different sources. Adjuvants like coconut water (5-20%) and casein hydrolysate (500-1000 mg/l) enhanced embryogenic callus growth (Armstrong and Green, 1985). Casein hydrolysate (CH) @ 3 g/l to the medium was effective for organogenesis in rice (Davayon, 1991). Larkin (1982) found that CH significantly shortened the lag period in the growth of sugarcane suspension cultures, though, it did not increase the rate of growth following the lag phase. Lago and Peteiro (1988) obtained high callus growth indices in sugarcane cultures grown in media containing myo-inositol or coconut water. Calli of sugarcane obtained from different varieties showed that inositol, thiamine and coconut water were effective in giving 100 per cent callus regeneration (Punce and Fuchs, 1989). Gonzalez *et al.* (1990) documented auxin like effect of coconut water in sugarcane cultures. Addition of source of nitrogen in reduced form, such as, yeast extract enhanced callus growth in maize endosperm culture (Tomes, 1985). Addition of activated charcoal to the media may be both beneficial or harmful. Mohamed Yaseen *et al.* (1995) and Detheir *et al.* (1993) noticed that embryogenesis could be induced in suspension culture of *Cenchrus ciliaris* by culturing on activated charcoal containing 2,4-D free medium. Supplementing potato extract and activated charcoal to the media used for rice anther culture was found effective by Kim *et al.* (1989) as callus induction was increased by addition of both. Yoshida *et al.* (1999) showed synergistic effect of proline with sorbitol. Claparols *et al.* (1993) investigated the effect of four exogenous amino acids, *viz.*, proline, glycine, aspargine and serine in the production of maize embryogenic callus production.

(d) Plant growth regulators

Growth regulator requirements for callus induction and successful plant regeneration are as varied as the explant and the genotype. Hormones are intricately associated with plant growth and development, yet their precise role in embryogenesis and organogenesis from cultured tissues is not fully understood. The mechanism of how plant cells perceive hormonal substances and translate the signals into a particular response are still largely unclear. There had been very little success in inducing callus and maintaining good growth of cereal tissue cultures in early stages (Norstog, 1956; Carew and Schwarting, 1958 and Tamaoki and Ullstrup, 1958). This problem was resolved by using higher concentration of auxins in the media for cereals (Carter *et al.*, 1967 and Yamada *et al.*, 1967). Auxin is one of the most significant key regulators for dedifferentiation and redifferentiation in monocot plants (Nishi *et al.*, 1968).

Many authors worked with 2,4-D and satisfied with its performance in case of monocot tissue culture. 2,4-D was used @ 2mg/l by ElWaffa and Ismail (1999) in wheat. In rice, according to Maheswari and Rangasamy (1989), MS medium containing 2,4-D (2.0 mg/ml) combined with 0.5 mg/l kinetin was effective for callus induction. Kucherenko (1993) found successful callus induction when 2,4-D was added to the medium. Hua *et al.* (1996) observed that hormones significantly affected callus induction frequency and callus quality in rice. 2,4-D revived plant regeneration in recalcitrant calli of rice when grown on for 20 days (Kishor *et al.* 1999). Cell proliferation was induced by 2,4-D at concentration 0.5-6.0 mg/ml as noted by Sarsenbaev *et al.* (1988). According to Taniguchi *et al.* (1991), the growth of embryogenic callus of barely was promoted by increasing 2,4-D concentration.

High concentration of 2,4-D was not always good for cultures as observed by Kuusiene and Sliesarvicius (1991). Although they found high frequency of callus formation at the concentrations of 10-15 mg/l of 2,4-D, yet the regeneration frequency obtained was highest at lower 2,4-D concentration (2.5-5.0 mg/l) in *Festuca pratensis*. 2,4-D alone was not as effective as in combinations with other growth regulators. Medium supplemented with a

mixture of 2,4-D, NAA and kinetin was more effective than that containing single plant growth regulator (Zhang, 1991). Hassawi *et al.* (1990) established on triticale that anther culture media supplemented with 2 mg/l IBA + 1 mg/l kin were most suitable for mature and direct plantlet development. Callus production was best with 2 mg/l 2,4-D and 1 mg/l kinetin. On the contrary, Furine and Jewell (1994) proved 2,4-D to be significantly superior for stimulating and maintaining embryogenic calli.

Some times 2,4-D at the different concentrations suppressed direct formation of green plantlets (Dhia *et al.,* 1990). Callus culture could not be maintained on media containing 2,4-D for period longer than three months while dicamba was useful for long term maintenance of culture. N_6 medium supplemented with 2 mg dicamba/l proved to be better by Bohorova *et al.* (1995) than any other media tested for callus initiation and maintenance from maize inflorescence.

Barro *et al.* (1999) found that regeneration from embryogenic calli induced on medium with picloram was almost twice as different as regeneration from cultures induced on 2,4-D in wheat and *Tritordeum* in case of inflorescence derived calli while for scutella highest frequency of embryogenesis and regeneration was found with 2.0 mg/l 2,4-D and the amino acids in half strength.

Different doses of abssicic acid (ABA) have been tried for inducing of embryogenic cultures in cereals. Carman (1989) noted decreased rate of embryogenesis in immature embryo explant of wheat when ABA was used with IAA or zeatin. Rafi *et al.* (1995) observed callus induction and proliferation were arrested by ABA treatment in first month. However enhanced growth of calli, up to three folds, was observed in ABA treated calli as compared to untreated controls. Increased plantlet production was found when ABA was incorporated in the medium. ABA was also effective for inducing pollen embryogenesis of rice (Sohn *et al.,* 1996).

2.2.1.2 Type of callus

The establishment of callus capable of regeneration and continuous growth in periodic subcultures depends to a large extent on culture conditions and the origin

of the explant. The callus may be compact or loose and friable, white, green, greenish yellow or deep red. Embryogenic callus (E callus) referas to callus which has the potential to regenerate plants. This term includes regeneration via both organogenesis and embryogenesis as long as regeneration can be maintained over a prolonged period of time. Non-embryogenic callus (NE callus) refers to callus which has little or no probability of regenerating plants either by organogenesis or embryogenesis or germination of pre-existing meristem (Tomes, 1985).

A distinctive feature of embryogenic cell culture in cereals is their colour and surface morphology. The appearance of the compact, nodulated callus that is milky white to yellow in colour is the firat visible indication of embryogenic callus development in most cereals (Bhaskaran and Smith, 1988). NE callus is loose, crystalline and yellow to brown in colour. (Nabors *et al.*, 1983 and Abe Futsuhara, 1985). Heyser *et al.* (1983) were able to distinguish between embryogenic and non-embryogenic rice calli by differential shoot initiation at low frequency from non-embryogenic callus type. Two types of distinct callus were observed by Gupta *et al.* (1989) in rice, E callus composed mostly of isodiametric cells which were yellowish and compact with organised globular structures and NE callus composed mostly of tubular cells which were white, unorganized and soft.

Kucherenko *et al.* (1988) categorized all types of rice calli into four main groups differing in morphogenetic potential (highly regenerative, unregenerative, regenerative and intermediate). Selection of callus on the basis of these traits enabled regenerates to be obtained from callus subculture from 14 passages (450 days), whereas without selection, callus normally retained morphogenetic potential for not more than 3-4 passages (90-120 days). In sorghum unorganised, translucent, fast growing, nonmorphic, nonembryogenic callus had a short life span and was not capable of plant regeneration (Kurvinashetti *et al.*, 1998 and Lusardi and Lupotto, 1990). Pan and Liang (1991) showed that rapid callus growth gave poor quality callus. High callus induction frequency was not always associated with high regenerative ability.

E callus contains abundant starch, which gives it the white colour (Karlsson and Vasil, 1986 and Close and Ludeman, 1987). The presence of starch has been reported in E callus of sorghum (Bhaskaran *et al.*, 1988). Vasil (1988) noticed that

loss of starch grains in *Pennisetum* cultures led to irreversible differentiation or senescence of embryogenic callus into NE cells.

Embryogenic calli were generally organised, white and with high morphogenetic sectors. Shoot were successfully obtained only from organised callus (Kuruvinashetti *et al.*, 1988). These calli were firm white and had nodular surface in rice (Marassi *et al.*, 1993). Wheat cultivar showed an off-white, compact and nodulated callus and a whtie, compact callus termed as E callus (Redway *et al.*, 1990 and Varshney *et al.*, 1999). They were rich in endoplasmic reticulum, polysomes and small protein bodies and the outer most layer of their cell wall was composed of fibrillar material. Samaj *et al.* (1999) noted that embryogenic units of friable maize callus were formed as globular or oblong pockets of tightly associated meristematic cells. These units were surrounded by conspicuous cell walls.

The nature of the callus tissue, its texture, compactness, friability and colour depend on the genotype, age of explant and even the season. With ageing of callus, islets of meristem were formed (Bhaskaran and Smith, 1990 and Narayanswamy, 1994). In pearl millet, a compact pale yellow callus arose from the peripheral calls of the scutellum and from the young inflorescence. It formed embryoids on transfer to certain media (Vasil and Vasil, 1981). Lu and Vasil (1981) recognised two types of cells in suspension culture of *Panicum maximum,* small, richly cytoplasmic and often starch containing E cells and large vacoulated NE cells.

A wide range of media-supplements affect the type of calli formed. Armstrong and Green (1985) indicated that addition of proline to the media increased the formation of E cells. The endogenous free proline content in embryogenic maize callus was significantly higher than in NE callus regardless of the presence of proline in the medium (Claparobs *et al.*, 1993). In maize, increased osmoticum, high sucrose or ABA treatment also produced the smooth, knobby E callus (Close and Ludeman, 1989). High sucrose promoted E callus formation (Vasil *et al.*, 1983). Frequency of embryogenic callus formation was related to the concentration of plant growth regulators in explant (Dolgykh *et al.*, 1999).

2.2.2 Morphogenesis

Morphogenesis refers to the origin of form and by implication, the differentiation of associated internal structural features. Plant regeneration from cultures occurs via organ differentiation, somatic embryogenesis, or both. Organogenesis in the form of root seldom leads to viable plants whereas shoot forming cultures have been able to produce viable plants by subsequent rooting.

There are many tissue culture systems where both embryogenic callus and shoot buds were produced from the same culture (Vasil *et al.*, 1985). Some of the bud like structure were considered to be precociously germinating embryoids (Vasil, 1987).

2.2.2.1 Organogenesis

Organogenesis can be defined as the origin of shoot bud and roots from explants either directly or via callus phase. Organization can be brought about in callus by the controlled initiation of an organ primordium through manipulation of the nutrient and hormonal constituents in culture media. Each tissue has its own requirements and some times less defined growth adjuvants play a part in organogenesis.

Shoot organogenesis is characterized by the production of a unipolar bud promordium with subsequent development into a leafy vegetative shoot. The developing shoot induces procambial strands to establish a conducting connection between the young shoot and the maternal tissue. The shoot then becomes rooted via root primordia formation and subsequent root organogenesis (Brown and Thorpe, 1986).

Factors affecting organogenesis

(a) Genotype

Genotypic differences were observed in shoot differentiation and maintenance of regeneration capacity by Cai and Butler (1990), and Kosting *et al.* (1996) in sorghum; Hanzel *et al.* (1985) and Dehleen (1999) in barley; Rajyalakshmi *et al.* (1988) and Mathias (1988) in wheat; Green (1977) in corn and Cummings

et al. (1976) in oats. In oats, the genotype is an important factor for plant regeneration. Cumming *et al.* (1976) found that out of twenty-four genotypes nine could be regenerated while five had a very low frequency of plant regeneration.

Although all cultivars of wheat have the potential to form embryogenic callus, the specific genotypes have an important role in determining the induction frequencies of callus and their morphology (Sears and Deckard, 1982; Maddock *et al.*, 1983 and He *et al.*, 1988). Dorosiev *et al.* (1991) noted that callus formation and regenration were dependent on genotype and ploidy level.

(b) Explant

Organogenesis is generally dependent upon the explant (Kasperbauer, 1990). The size of the explant cultured had significant effect on regeneration. Loss of regenerability has been observed in the smaller explants, while the larger explants consisting of parenchyma, vascular tissue and cambium showed spontaneous initiation of shoots buds irrespective of the auxin-cytokinin concentrations (Okazawa *et al.*, 1967).

In immature embryo cultures of maize, proliferation of cells in the nodal region of the embryo axis gave rise to compact callus at high sucrose levels and leafy structures at low sucrose levels (Vasil *et al.*, 1983). Feroughi *et al.* (1979) obtained initiation of new centres of embryo development when wheat florets with immature embryos were sprayed with 2,4-D.

(c) Media and Media Adjuvants

During organogenesis, separate culture media are usually used for shoot and then root formation. Certain cereals may require modification of the MS salt to satisfy nutrient requirement (Phillips and Collins, 1979).

Koetzi *et al.*, (1989) found that plant regeneration was influenced by organic supplements to the basal media, medium solidifying agents, and phytohormone concentration during callus induction. Regeneration was accomplished by subculturing to identical medium lacking 2,4-D. The influence of the total nitrogen content, the NO_3- and NH_4+ ratio and ratio of organic and inorganic nitrogen was determined by Mordhorst and Lorz (1993).

Copper sulphate significantly enhanced shoot regeneration as observed by Purnhauser and Gyulai (1993). MS was found better than B_5 for plant regeneration by Gamborg *et al.* (1977). The effect of 2,4-D on plant regeneration has been reported by many authors. Heinz and Mee (1969) reported regeneration of plants from callus cultures established from shoot apices, leaves and inflorescence of *Saccharum* species. The callus cultures were induced on MS medium supplemented with 136μM2,4-D and 10 per cent (v/v) coconut water. Transfer of callus to a medium without 2,4-D produced plant differentiation.

Ahloowalia (1975), Cheng and Smith (1975), Sangwan and Gorenflot (1975), and Green and Phillips (1975) reported plant regeneration from callus on medium with 2,4-D and reduction or removal of 2,4-D from the medium. Rye grass callus was induced from immature seeds on MS medium supplemented with 0.8 μM 2,4-D, 3.7 μM IAA and 16.0 μM kinetin and plant regeneration occurred on half strength MS media with 3.4 μM 2,4-D, 21.4 μM IAA and 5.0 μM kinetin. Addition of coconut water promoted shoot organogenesis (Ahloowalia, 1975).

The possibility of increasing the frequency of the regenerants by optimizing the growth regulators, composition of the medium for morphogenesis and repeated induction of regenerants from morphogenetic callus was tested by Davoyan (1991). The highest frequency was obtained by subculturing callus on medium supplemented with 2,4-D (0.5 mg/l) and BA (5mg/l). the medium for organogenesis was most effective when it contained casein hydrolysate (3 mg/l). Freshly prepared medium surpassed conditioned medium in the frequency of regenerants.

Fekete and Pauk (1989) found significant increase in plant regeneration by using 2,4-D (1-1.5 mg/l) and kinetin (0.5-4.0 mg/l) in various combination at the first stage, followed by transfer of embryogenic callus to medium containing 1 mg/l kinetin in wheat.

Doroseiv *et al.* (1991) established in barley that regenerative ability was heighest at 2,4-D concentration of 2 mg/l and lowest at 6 mg/l. regeneration was dependent on the content of 2,4-D present in media. The morphogenetic

potential of the callus of diploid varieties was inversely proportional to the 2,4-D concentration.

Cytokinins are known to enhance multiplication of buds on cultured tissue (Bhaskaran and Smith, 1990). However exogenous cytokinins were not always required in all cases for regeneration, probably because of adequate levels were already present in some tissues (Norstog, 1970; Henke *et al.*, 1978 and Inoue *et al.*, 1979). The tissues that did not contain adequate levels were required to supplied with exogenous cytokinin. Papenfuss and Carman (1987) obtained enhanced regeneration from wheat callus cultures using kinetin.

Patel *et al.* (2001) examined the effects of different levels of kinetin, BA and coconut water. These three contents had marked effect on shoot multiplication. The lowest multiplication ratio was recorded in treatment combinations comprised of lower concentration of kinetin and BA. In barley, callus induction occurred from apical meristems on MS medium with 10 μM IAA, 15 μM 2,4-D and 1.5 μM 2-ip whereas plant regeneration was obtained on the same medium without these growth regulators (Cheng and Smith, 1975).

Srivastava and Chawla (2001) observed low frequency of regeneration on the medium without any growth regulator. There was a 14 per cent increase in regeneration frequency with the increase in cytokinin concentration and levels of auxin when compared with media containing only cytokinin.

(d) Stress

Osmotic stress has been reported to play an important role in morphogenetic potential of regenerating calli by various workers. ElWaffa (1999) observed in wheat that the proliferation of embryogenic calli declined markedly with increasing osmotic stress.

A high frequency of plantlet formation in indica rice was obtained when calli were regenerated on a medium supplemented with 10 μM kinetin and 5 μM NAA solidified with 1.6 percent agar instead of 0.8 per cent, which is normally employed (Gandhi and Khurana, 1999). The simple water stress enhanced the frequency of plantlet regeneration even in the absence of hormone. The stress treatment was also effective in other indica genotypes of rice which normally

regenerated poorly on basal or hormone supplemented media and promoted shoot regeneration from long term callus cultures.

Significant increase in green plant regeneration was recorded by Immonen and Anntila (1999) when cold pretreatment of 2-4 weeks in barley was applied. Dim light during the stress period improved green plant regeneration. Regeneration was more dependent on the average solar radiation than on temperature. Plant regeneration was higher and less variable in plants grown in growth chamber.

(e) Age of culture

Many tissue exhibit a high potential for organogenesis or embryogenesis when first initiated, but gradually this potential declines with the proceeding subcultures with eventual loss of morphogenetic response. Several workers have observed this phenomenon in callus that had passed through several passages (Murashige and Nakano, 1967 and Torrey, 1967). Regeneration ability from scutellum and plumule of rice decreased with increasing subcultures (Kucherenko *et al.*, 1988). Binh and Heszky (1990) showed no regeneration from fine and friable callus cultures of rice after two years of its maintainance. Increase in the number of subcultures leading to decreased regeneration was also noted by Gao-Zhen *et al.* (1999).

The loss of morphogenetic potential in a tissue may be due to either a genetic or a physiological change induced by prolonged cultural conditions. The genetic effects in a cultured tissue are reflected in changes of chromosome number leading to euploidy or aneuploidy mutations. A correlation between changed ploidy and loss of regenerative potential has been noted in several callus tissues (Murashige and Nakano, 1967 and Torrey, 1967). According to Smith and Street (1974) subculture leads to the cells impared or of no totipotency due to some changes in nuclear cytology, and such cells are at selective disadvantage as compared to the normal cells. Eventually at later stages of cultures, they are entirely replaced by nontotipotent cells.

The addition of cytokinin partially restored the potential for shoot organogenesis. Pius *et al.* (1993) successfully delayed the loss of regeneration in embryogenic cultures of pearl millet by using ethylene inhibitors.

2.2.2.3 Root Differentiation

During organogenesis, separate culture media have been used.generally for shoot and then root formation. Cytokinin is necessary for shoot formation while an active auxin or reduction in the cytokinin level is required in successful rooting media.

Oxygen gradient in a tissue culture may play an effective role in the promotion of organogenesis as confirmed by Kessel and Karr (1972) in carrot. With reduction in the available oxygen, shoot formation was favoured, while rooting required an increased oxygen gradient.

Root formation had been a fairly easier task in most of the grasses with the exception of sugarcane. Generally a medium with or without a lower concentration of any auxin, such as IBA or NAA was useful in most of the cereals (Bhaskaran and Smith, 1990). Chin and Scott (1977) proposed that NAA (1.0 mg/l) was effective in inducing roots from wheat calli. The quality and intensity of light often played a key role in the phenomenon of organogenesis (Weis and Jaffe, 1969). The blue region of the spectrum promoted shoot formation and red light favoured rooting (Letouze and Beauchesne, 1969). Barba *et al.* (1981) suggested that shoot exposed to higher light intensity would develop a profusely branched root system, more vigorous stand and better survival on transfer to soil in sugarcane. Bhansali and Singh (1982) obtained adventitious roots from sugarcane in MS media with NAA @ 5 mg/l or IAA @ 7.5 mg/l.

Rodriguez (1982) induced differentiation of shoots and roots from sugarcane calli in two weeks by eliminating 2,4-D and coconut water from the medium and by providing constant illumination. Martzki and Hiraki (1980) documented promotion of root formation in *Saccharum* species by increased sucrose level. Grisham and Bourg (1989) found half strength MS salt with 6 per cent sucrose in liquid media to be favourable for inducing roots in sugarcane, whereas Diaz *et al.* (1989) demonstrated full strength MS salts with IAA and 4 per cent sucrose to be the best root induction medium in shoot tip cultures. Telgen *et al.* (1992) indicated that even at low concentration of growth regulators the shoot would root, but the presence of kinetin may hinder the development of lateral roots. Purnhauser and Gyulai (1993) triggered root formation by using copper sulphate while silver nitrate inhibited rooting in wheat and triticale. Kharinarain *et al.* (1996) showed

that SH medium with 5 mg/l NAA promoted rooting. Plantlets were rooted on MS media supplemented with 0.5 mg NAA/l in wheat (Varshney *et al.,* 1999).

2.2.2.3 Somatic embryogenesis

Somatic embryos are induced from callus by a relatively simple manipulation of culturing conditions. The overall embryogenetic potential of a callus is highest when it is relatively young and resides with a subpopulation of the culture called "proembryogenic masses" (Zimmerman, 1993).

The phenomenon of somatic embryogenesis in cereals and grasses is rare (Gamborg *et al.*, 1970; Norstog, 1970 and Vasil and Vasil, 1981). The formation of somatic embryos which are said to be of single cell origin (Haccius, 1978), is of rare occurrence in cereals. Regeneration of plant via somatic embryogenesis is preferred to organogenesis because embryo usually arise from single cells (Backs-Husemann and Reinert, 1970; Dunstan *et al.*, 1978 and Vasil, 1988). However, Wernicke *et al.* (1982) presented evidence that somatic embryos are of multicellular in origin.

The first report of somatic embryogenesis in cereals was made by Norstog (1970) who observed the development of single embryoids in the region of scutellar nodes of young barley embryoids.

The cells in compact calli which were differentiated and obtained embryogenic competence during the early stages of culture, could be perpetuated by subculturing and gave rise to embryoids and plants (Vasil and Vasil, 1981). Haydu and Vasil (1981) showed that the induction and isolation of a compact, white and organized callus tissue was the most critical factor in obtaining somatic embryogenesis *in vitro*. Bregitzer *et al.* (1991) isolated somatic embryos from friable and embryogenic callus of oats.

Embryo differentiation is considerably influenced by the physiological state of calli and the carry over effects of auxins from inoculum to subculture. Nutrient media used for initial proliferation of tissues played a vital role in inducing embryogenesis. Cytokinin and gibberellins caused a partial or complete inhibition of potentially embryogenic cells. 2,4-D in the medium was least conducive for either organogenesis (Lustinec and Horak, 1970) or embryogenesis (Reinert and

Backs, 1968) but its effects are more pronounced when applied in sequence (Reinert and Tazawa, 1969).

Halperin (1970) has shown that embryogenesis could be induced only in those suspension cultures which are derived from explants grown on auxin-containing media.

Differentiation of somatic embryos depended on auxin concentration (Kamiya *et al.,* 1988; Talwar and Rashid, 1990; Garcia *et al.,* 1991; Cheng, 1992; Dolgykh *et al.,* 1999 and Lambe *et al.,* 1999).

Shoot differentiation follows the transfer of callus tissues from a medium containing 2,4-D to medium devoid of synthetic auxin or containing its very low concentration. Cheng (1992) observed in *Pennisetum purpureum* that somatic embryos were developed on the media containing a low concentration of 2,4-D.

Dolgykh *et al.* (1999) showed in maize that tissues with competence for somatic embryogenesis were characterized by low levels of auxin and cytokinins and higher level of ABA.

Somatic embryogenesis was most frequent on medium containing 0.5 mg/l to 0.1 mg/l 2,4-D with or without kinetin. Somatic embryos on medium with 2,4-D showed germination or fused together to form callus (Garcia *et al.,* 1991). High concentration of auxin disrupted the organization of embryoids *in vitro*, resulting in the formation of a nodular callus (Kohlenbach, 1978). Under the influence of auxin in the medium, scutellar cells of immature embryo of pearl millet generally gave rise to an embryogenic callus instead of directly forming embryoids (Vasil and Vasil, 1981).

Lambe *et al.* (1999) observed in pearl millet that the transfer of embryogenic calli onto auxin free medium was sufficient for inducing somatic embryo development in short term cultures. Maturation of embryogenic calli on medium supplemented with activated charcoal, followed by the germination of somatic embryos on medium supplemented with gibbererellic acid, restored regeneration in long term cultures.

Subhadhra *et al.* (1995) found somatic embryoids in cell suspension of wheat when they reduced the concentration of 2,4-D. These embryoids gave normal plants only in the presence of reduced concentration of 2,4-D.

Talwar and Rashid (1990) demonstrated that differentiation of somatic embryos of *Pennisetum typhoides* depended on auxin concentration and the minerals in the medium. Low level of 2,4-D in N_6 media with a low level of ammonium nutrient favoured the formation of somatic embryos while on MS medium containing high ammonium, compact callus was formed.

High frequency of regeneration of plantlet through somatic embryogenesis was obtained by Bhat and Kuruvianashetti (1994) on MS medium supplemented with 0.5 mg/l BAP.

The form in which nitrogen is supplied to the tissue system is another factor in the determination of somatic embryogenesis. Halperin and Wetherell (1965) have observed that ammonium ions and casein hydrolysate at low levels were more stimulatory to embryogenesis in comparison to nitrate. On the contrary, use of 1 g/l casein hydrolysate and 164.8 μM mannitol resulted in the reduction of somatic embryos (Suprasanna *et al.*, 1997).

Significant increase was found by Sargent *et al.* (1998) in plant regeneration from somatic embryogenic callus of sorghum and sugarcane following induction on media supplemented with either putrescine, spermine or spermidine. Ethylene biosynthesis significantly decreased the percentage of explants that underwent embryogenesis while it was increased in response to the addition of polymines.

The induction of compact callus and embryo like structures was promoted by addition of sorbitol to callus induction media (Ryschka *et al.*, 1991).

Rout *et al.* (1998) found an increase in the efficiency of somatic embryogenesis in leaf base and mesocotyl derived calli of *Setaria* by the use of nickel. A lower concentration of nickel in the culture media promoted long term maintenance of embryogenic calli that regenerated into plantlets

2.3 Tissue culture studies in *Cenchrus* species

The first documented report on the *in vitro* studies in *Cenchrus species* was in *Cenchrus ciliaris* (Sankhla and Sankhla, 1989) followed by another investigation in *C. ciliaris* and *C. setigerus* (Kackar and Shekhawat, 1991).

Young immature inflorescence explants were most preferred for callus induction in the initial studies (Sankhla and Sankhla, 1989 and Kackar and Shekhawat, 1991), though the frequency of regenerable calli and plant regeneration were not documented. However, Ross *et al.* (1995) preferred to use mature seeds as a more readily available source of explants since recognizing appropriate developmental stage of immature inflorescence or embryos was difficult in *Cenchrus* species.

Similar to the other monocotyledons, callus induction in *Cenchrus* species was tried with varying concentrations of 2,4-D (1.0 to 20.0 mg/l) in combination with other growth regulators such as IAA and Kinetin (Sankhla and Sankhla, 1989) or with media adjuvants like ascorbic acid (Kackar and Shekhawat, 1991) or coconut water (Ross *et al.,* 1995) on MS medium. According to the later workers 2.5-6.0 mg/l 2,4-D for *C.ciliaris* and 4.0-14.0 mg/l 2,4-D for *C. setigerus* were optimal for callus induction and maintenance. Earlier, Sankhla and Sankhla (1989) could initiate callus production using 1.0 mg/l 2,4-D with 5 mg/l IAA and 0.5 mg/l kinetin. The role of ascorbic acid as an essential anti-oxidant in callus culture of *C. ciliaris* has been emphasised (Kackar and Shekhawat, 1991). Addition of 5 per cent coconut water was also beneficial for raising calli (Ross *et al.,* 1995). The types of calli in *C.ciliaris* and *C. setigerus* could be mainly distinguished into a slow growing, hard, nodular and white compact embryogenic callus (Sankhla and Sankhla, 1989; Kackar and Shekhawat, 1991 and Ross *et al.,* 1995) and a rapidly growing friable watery textured non-embryogenic callus (Kackar and Shekhawat, 1991 and Ross *et al.,* 1995). However, Ross *et al.* (1995) also found a third type of callus characterised by yellowish and relatively compact texture, often containing shiny mucilaginous cell. All the workers have concluded that only the first type of callus was embryogenic and profusely regenerating.

Mostly the regeneration of plantlets from calli was through somatic embryogenesis. All the earlier workers found that transfer of calli to hormone free MS medium could induce the formation of proembryoids at varying degree, while prolonged maintenance of callus in same induction medium resulted in gradual appearence of proembryoids (Sankhla and Sankhla, 1989). Addition of coconut water as adjuvant could produce proembryoids in 20 to 50 per cent of the calli (Ross *et al.,* 1995) in *C. ciliaris*. Rooting of regenerated shoots from the embryoids

occurred on hormone free media. Nevertheless, use of 0.1 mg/l NAA in the medium was helpful for root development and easier acclimatization of the regenerants (Kackar and Shekhawat, 1991). Gradual hardening of the plants and their survival has also been established by these investigations. However, no comprehensive data on optimization of culture medium and conditions, amenability of different explants and genotypes for *in vitro* response and the possibility of enhancing the efficiency of regeneration in a variety of situations have been available in *C. ciliaris* and *C. setigerus* and also that there is no report so far available on tissue culture studies in *C. echinatus*.

2.2.4 Hardening

It is recommended that removal of sugar from the supporting medium, preconditioning to low relative humidity, high light intensity and high temperature could ensure higher survival rate during transfer of plantlets to natural conditions. The gradual removal of sugar has been suggested to stimulate photosynthetic ability.

In wheat, the percentage of surviving regenerants and of the fertile plants depended on genotype (Rakhimbaev and Kushnarenko, 1991). Copper sulphate pretreatment promoted plant survival when regenerated wheat plants were transferred directly to soil (Purnhauser and Gyulai, 1993)

Shukla *et al.* (1994) indicated that plant survival in sugarcane was almost 100 per cent in hydroscopic system and 80-90 percent in potting mixture of farmyard manure, sand and soil. *In vitro* selection reduced regeneration frequency and subsequent survival of sorghum plants under field stress (Duncan *et al.*, 1995). Plantlets of *Cenchrus ciliaris* were transferred to field after acclimatization in growth chamber at 26^0 C (Kackar and Shekhawat, 1990).

2.4 Somaclonal variation

Regeneration of plants from protoplast, cell and callus cultures provides a novel method to produce new types. Larkin and Scowcroft (1981) coined the term **somaclonal variation** to cover phenotypic changes which occurred in plants

regenerated from cultured cells or tissues. The cause of somaclonal variation still remains uncertain. Genotype (Felfoldy and Purkhauser, 1992) and duration of the callus phase (Wenzel, 1988) together with the genetic changes during callus phase have been considered to be the important factors for somaclonal variation. Useful variability could be generated without sexual recombination in somaclonal variation (Larkin, 1987).

Tissue culture regenerated variants have also been called calliclones (Skirvin and Janicks, 1976), phenovariants (Sibi, 1976), protoclones (Shepard *et al.,* 1980), subclones (Cassells *et al.,* 1991) and gametoclone (Evans *et al.,* 1984). Somaclonal variations have been observed and discribed in a large number of plant species across numerous genera and families including crop plants. All plant species have shown somaclonal variation at various levels to varying degrees, when cultured *in vitro.* The variations have been of both qualitative and quantitative nature, verifiable at phenotypic, biochemical and molecular levels (Peschke and Phillips, 1992). Novel variants have been reported among somaclones and genetic and cytogenic evidences (Compton and Veilleux, 1991; Kaltsikes and Bebeli, 1993 and Puolimatka and Karp, 1993) indicate that both the frequency and distribution of genetic recombination events could be altered by passing through tissue culture. This suggests that variations may be generated from different areas of the genome than those that are accessible to conventional and mutation breeding. Somaclonal variation has been most successful in the crops having limited genetic recombination systems (*viz.*, apomictic, vegitative reproducers) and /or narrow genetic base (Karp, 1995).

For genetic analysis of somaclones, Orton (1984) recognized the need for unified nomenculture and introduced the R_0, R_1, R_2 etc., respectively. Larkin and Scowcroft (1981) referred to the regenerated plants as SC_1 plants and subsequent self-fertilized generations as SC_2, SC_3, SC_4 etc. Larkin *et al.* (1984) proposed this terminology equivalent to R_0, R_1, R_3 etc., respectively as proposed by Orton (1984).

A major challenge for exploitation of somaclonal variation is the identification of useful variation (Jones, 1990 and Smith *et al.,* 1993). Since a large number of cells

in cultures are generally variable, they are not required to be exposed to *in vitro* selective agents to develop novel somaclonal variants.

The principle criterion necessary for improved genotype selection using this technology would depend on expression of a stable target genetic trait while other desirable traits remain unchanged by screening large population of cells *in vitro* (Smith *et al.,* 1993).

2.4.1 Spectrum of somaclonal variation

Somaclonal variations might be generated for various morphological and agronomic traits (Mohumand and Nabors, 1990). Somaclonal variations among progenies of regenerants for numerous morphological and agronomic traits have been reported, such as for plant height (Pei *et al.,* 1996 and Abbasi *et al.,* 1999), sterility (Garcia *et al.,* 1994; Chauhan and Singh, 1995 and Dolygykh, 1999), biomass (Bhaskaran *et al.,* 1987), grain yield (Mohumand and Nabors, 1990 and Diao *et al.,* 1999), tillering capacity (Hashim *et al.,* 1990 and Gasper *et al.,* 1995) and isozyme instability (Humphreys and Dalton, 1991 and Garcia *et al.,* 1994).

Abbassi *et al.* (1999) found significant reduction in plant height and days to flowering in rice. A linear relationship existed between increased L/B ratio and improved grain quality. Some differences for agronomic traits were also found in rice plants regenrated from seed versus plumule cultures. (Heszky *et al.,* 1989).

2.4.2 Factors affecting occurrence of somaclonal variation

The somaclonal variation arise in unpredictable and uncontrolled manner and hence are difficult to direct. A major advance in this respect has been the identification of internal and external factors that affect the nature and frequency of instability. The study of these factors may help in exercising some control over somaclonal variation. (Elkonon *et al.,* 1994 and Skirvin *et al.,* 1994).

(a) Explant source tissue

Few studies have focused on the influence of the explant source on somaclonal variation since the stages of development and organization of different plant organs have been considered to be crucial for the occurrence of somaclonal

variation. The meristem tip cultures were found to be comparatively free from somaclonal variation while the cultures of dedifferentiated explants invariably led to some variants. The meristem cultured without a state of differentiation produced little or no variation compared to when a differentiated state was induced (Potter and Jones, 1991).

Differences in the stability of tissue cultures produced from different explant tissue sources could often be traced to variability pre-existing in the explants. The most widely recognized case of this occurrence is polysomaty which is found in over 90 per cent of plant species.

Chauhan and Singh (1995) observed in wheat that variation for different plant morphological traits was greatest among regenerants derived from coleoptile nodes as compared to those from other explants.

Some differences in variation for agronomic traits were observed in rice plants regenerated from seed versus plumule cultures (Heszkh *et al.*, 1989).

Explant sources vary in their ability to generate somaclonal variation (Skirvin *et al.*, 1994). Highly differentiated tissues (roots, stem and leaves) produce more variation than explants with pre-existing meristems (Gui *et al.*, 1993).

Somaclonal variations result from both pre-existing genetic variation within the explants and variation induced during the tissue culture (Evans *et al.*, 1984). Variations may represent pre-existing variation or variation induced during callus formation (Skirvin *et al.*, 1994). The selection of novel genotypes with stable, heritable mutations could be achieved through *in vitro* cultures. Cytological studies indicated that explant regeneration was associated with genetic architechture (Karp, 1991). This resulted in a wide range of phenotypic variation in the regenerated plants. Variability in regenerated plants was higher among polyploid and high-chromosome number explant sources (Skirvin *et al.*, 1994) than among lower ploidy and low chromosome number species or explant sources.

(b) Culture type and mode of regeneration

Comparison of cytological instability in morphogenetic and non-regenerative callus indicated that while chromosome variation was present in both, it was far more prevalent in non regenerative callus (Singh, 1986). This has clear

implication on the degree of somaclonal variation recoverable in regenerated plants and may be one way in which selective constraints could be imposed.

Another important aspect has been the mode of regeneration or development pathway (organogenesis or somatic embryogenesis). Vasil (1986) argued that somaclonal variation in gramineae is largely confined to regeneration from organogenic cell cultures which contain large vacuolated, starch free cells and where plants are formed by the organization of shoot meristem. Plant regeneration by somatic embryogenesis are useually less variable than plants regenerated via shoot and root morphogenesis (Lorz *et al.,* 1988). In organogenesis, shoots and roots evolve from dedifferentiated calli and develop independently with respect to localization and timing (shoot preceding roots) while somatic embryos have a bipolar structure in which shoot and root meristems are directly connected with no interruption by nondifferentiated callus tissue (Lorz *et al.,* 1988).

Plant regenerated by somatic embryogenesis contain fewer mutation than those regenerated by organogenesis primarily because the embryoid is generally derived from a single cell (Vasil and Vasil, 1981) or from a small group of cells (Vasil *et al.,* 1985). Furthermore, the plants regenerated from soamtic embryoids contain few mutation or chimeras due to stringent internal genetic controls imposed during embryoid formation causing selection pressure against abnormal types (Swedlund and Vasil, 1985). However, many reports have documented somaclonal variation through somatic embryogenesis also (Karp, 1991). Enhanced variability from embryogenically regenerated plants from the same explant source have been documented.

A comparative study indicated that maize plants regenerated from embryogenic callus were more variable, both phenotypically and cytologically, than those from organogenic callus from the identical explant (Armstrong and Phillips, 1988).

(c) Culture age/duration of tissue culture phase

Many investigations have noted an increase in variability of all types with an increase in age of cultures (Armstrong and Phillips, 1988; Muller *et al.,* 1990 and Symillides *et al.,* 1995). This could be attributed to the culture *per se*

becoming more prone to the changes as it became older. The mutations induced in early cultures that increased in number over time or increased polyploidy.

A study by Benzoin and Phillips (1988) on maize indicated that the mutation rate did not necessarily increase over time but rather mutations occurred early that were able to accumulate and selection did not eliminate them. Evidence for sequential accumulation of independent mutations has also been described for four gene mutations in rice plants regenerated from tissue culture (Fukui, 1983). However, Ruiz *et al.* (1992) found that somaclonal variation did not appear to be a very frequent event in plants regenerated from one to six months old culture of barley.

(d) Action of growth regulators

Contribution of plant growth regulators to somaclonal variation has been documented by several studies. Most plant growth regulators more specifically, 2,4-D and BA have been involved in tissue culture induced variability (D'Amato, 1985 and Shoemaker *et al.,* 1991). Regeneration competence in Gramineae may be rapidly lost during dedifferentiation (Ozias-Akins and Vasil, 1988). This loss may be due to the endogenous concentration of growth regulators (Vasil, 1987). Tissue culture induced variability may increase as growth regulator concentration increases (Skirvin *et al.*, 1994).

The primary event causing tissue culture induced variability could be due to cell cycle disturbances (Pescke and Phillips, 1992) caused by exogenous hormones' effects (Liscum and Haugartee, 1991) or nucleotide pool imbalance (Jacky *et al.*, 1983). Auxin may produce rapidly disorganized growth during callus induction leading to genetic instability through asyncronous cell division (Lee and Phillips, 1988). Increased thimidine may enhance chromosome breakage (Ronchi *et al.*, 1986). Sister chromatid exchange frequency may increase with low concentrations of 2,4-D (Dolezel *et al.*, 1987). 2,4-D concentration in excess of 2mg/l induced substantial increase in the frequency of sister chromatid exchanges in chromosomes of the cultured wheat cells (Murate, 1989). Tissue culture induced variation might be due to the influence on the genes involve in plant hormone metabolism as the genes controlling phytohormone signals are directly involved in plant regeneration (Henry *et al.*,

1994). In some polysomatic plants the division of endopolyploid cell was found to be dependent on the presence of kinetin in the medium (Torrey, 1961). Endopolyploid cells may undergo amitosis to produce aneuploid, polyploid or haploid cells (D'Amato, 1985). Yet another contribution of growth regulators to somaclonal variation might be by the induction of epigenetic changes which could upset of plant growth and development.

(e) Explant genotype

Cultivars vary in their ability to produce regenerable cultures. The differences in the genotypes of explants of the same species may be due to either pre-existing variation or varietal differences. Somaclonal variation is primarily due to such pre-existing variation or to new mutation. Varietal differences for somaclonal variation have been reported in maize (Zehr *et al.*, 1987), wheat (Mohmund and Nabors, 1990) and rye (Rakoczy-Trojanowska and Malpeszy, 1993). Differences may exist in the degree to which the tissue culture environment disrupts the cellular environment of a particular line.

2.4.4 Mechanism of somaclonal variation

Somaclonal variation results in the production of new genotypes with a limited change in the original genome. As a source of variation, somaclonal variation mimics induced mutations (Karp, 1995). Somaclonal variations have been associated with changes in chromosome number (polyploid, aneuploid) and structure (translocation, deletion, inversions) point mutation, DNA methylation (Brown *et al.*, 1991) qualitative and quantitative changes in DNA during differentiation and development, unstable genetic loci changes in cytoplasm and plastids and activation of transposons. Cell divisions in cultures are subjected to peculiar stress not encountered under natural conditions. Culture conditions are known to affect cell division cycles in plants and the disturbances in cell division cycle have been attributed to induce somaclonal variations (Gould, 1984). The late replicating nature of heterochromatin may pe-trub the cell cycle and result in enhanced chromosome breakage when cells are induced to divide under *in vitro* conditions of cultures (Lee and Phillips, 1988). Genomes with large proportion of

heterochromatin would give more somaclonal variation than the genomes which are largely euchromatic (Benzoin and Phillips, 1988).

2.4.5 Inheritance of somaclonal variation

In nearly all cases where extensive field trials on somaclones have been carried out, there were clear evidences that changes in agronomic trait have occurred as a result of *in vitro* cultures.

In rice, Marrasi and Rapela (1992) showed variation in shooting ability, husk color, length and width of grain, panicle length and percentage of husk in the evaluated somaclones. Some of these characters were transferred to a second generation, indicating some stable inheritance. The potential of somaclonal variation to contribute genetic variation of wheat improvement has been widely discussed by Villareal *et al.* (1999).

In wheat, the size of flag leaves, plant height, tillers/plant, spike length, awn length and seeds per spike differed significantly among regenerants of two selfed recurrent generations (SC_1 &SC_2) and between SC_1 and parent controls (Hashim *et al.*, 1990). Chauhan and Singh (1995) tested somaclones for karnal bunt resistance in R_0 generation. Resistance was inherited upto R_3 generation. Li *et al.* (1995) found heritable and stable variation in progenies of plants regenerated from cultured immature embryos. Ling *et al.* (1995) observed that stable variation was the most common type in the R_2 population, accounting for approximately 1/3rd of the total variation. Somaclones of high quality were selected by Hu *et al.* (1996) and multiplied for eight generations. Pei *et al.* (1996) demonstrated that plant height in the R_2 and R_3 generation were positively correlated. R0 regenerants of the maize inbred had a high growth rate and tillering capacity. Its selfed progeny showed several heritably stable variants (Gasper *et al.*, 1995).

Somaclonal variation expressed in plants regenerated from *Paspalum dilatum* include characteristics influencing drought and heat tolerance and could be permanently fixed and expressed in subsequent generations of this species due to its apomictic nature (Tischler *et al.*, 1993).

Variations regenerated through tissue culture of nine sorghum genotypes were found inheritable in the R_2 families and expressed in the R_3 plants (Qun *et al.*,

1996). The acid soil drought stress, super tolerant selections were located even in the R_7 generation. A variation frequency of 0.1-0.2% for stress tolerance and acceptable agronomic trait among the surviving somaclones provided an adequate number of phenotype with desirable agronomic characteristics and a high level of soil stress tolerance (Duncane *et al.*, 1995). However, in most of the cases it had not been possible to select improved variations either due to the variations were in negative direction or the positive variations were associated with some negative alterations and sometimes even the changes obtained in somaclones were not stable. Qureshi *et al.* (1992) observed that in spring wheat, higher grain protein levels in seed progenies of somaclones were associated with lowered yield. Similarly Baillie *et al.* (1992) observed that a field evaluation of somaclones of barley had little variation and that was of negative value.

2.4.6 Biochemical analysis in somaclones

Isozyme analysis has become particularly prominent in systematic and evolutionary biology as well as agronomy (Tanksley and Orton, 1983). Isozyme or multiple molecular forms of enzymes are the enzymes that share a common substrate but differ in electrophoretic mobility (Markert and Moller, 1959). For isozyme,a change must have occurred in base composition of enzyme that effects its migration in an electric field. Isozyme studies have been especially useful in tissue culture analysis otherwise somaclonal variation will go undetected (Shenoy and Vasil, 1992). Evans and Sharp (1983) and Lee and Phillips (1988) demonstrated that genetic variation in tissue culture derived plants could result from change in chromosome number and/or chromosome rearrangements. Brettell *et al.* (1986) showed molecular analysis of an electrophoretic mobility variant for Adh-1 isozyme in maize. They found that this variant was generated by a single base change in the Adh-1 gene sequence resulting in substitution of one amino acid for another.

The electrophoretic pattern of storage proteins of wheat and barley are very sensitive parameterss for assessing somaclonal variation (Larkin *et al.*, 1984). The somaclonal mutants recovered from tissue culture of hexaploid bread wheat had alterations in the expression of genes (Larkin *et al.*, 1984 and Maddock *et al.*,

1983). The progeny of 551 regenerats of the hexaploid wheat cultivar were analysed by Davies *et al.* (1986) for somaclonal mutants at the three Adh-1 loci. A simple electrophoretic assay allowed identification of null mutants or mutants rise to altered enzyme activity or electrophoretic mobility at each Adh-1 loci. Isozyme analysis of wheat somaclones has been an efficient method of recovering isochromosomes, translocation lines and aneuploids in any cultivar amenable to tissue culture.

Many workers have conducted isozyme analysis of tall fescue plants regenerated by tissue culture. Isozyme variation has been reported among individual tall fescue plants (Eizenga and Buckner, 1986) and among plants derived from anther-panicle cultures (Eizengo, 1987). Zymogram of the parents and regenerants were obtained by Dehleen and Eizenga (1990) for the enzyme ACP, ADH, GOT, 6PGD and PGI. Isozyme variation was observed for two groups of plants derived from the embryo. One group of four monosomic derived plants differ for the enzymes GOT and ACP and all four plants have a PGI pattern different from that of the parental monosomic plant. This indicated that the loss of PGI allele was probably as a result of callus culture. A direct relationship was found by Humphreys and Dalton (1992) in regenerated plants between time in cell suspension and the number of aberrations in PGI/2 locus. The PGI/2 locus was found suitable for studying genetic stability in cell cultures of *Lolium multiflorum* and *Festuca arundinacea* since it is highly polymorphic and allows for the differential labeling of both homologous and homeologous chromosomes by phenotypically distinct PGI/2 allels. Eizengo and Cornelius (1991) showed that among the isozymes they studied in *F. arundinacea,* PGI/2 was one of the most sensitive to culture condition. Gracia *et al.* (1994) noted that *in vitro* cultures might produce chromosome mutation and PGI/2 instability within a short period of time. Two genotypes showed instability at the PGI locus in tall fescue.

Shenoy and Vasil (1992) investigated the extent of biochemical and molecular variation in 63 plants of napier regenerated from 3-24 weeks old embryogenic callus cultures. The entire population was analyzed for the activity of fourteen isozyme systems but no qualitative variation was found at any of the loci

examined. These results confirmed that plant derived from somatic embryos were genetically uniform.

Amberger *et al.* (1992) observed variant isozyme pattern in two independent tissue culture derived lines. Genetic analysis was conducted on these two isozyme variant and they were heritable. No variants isozyme pattern was evident in control (Parental) soybean lines. The two mutant phenotypes, chlorophyll-deficient and Mdh null (mitochondrial malate dehydrogenase) were found to cosegregate. The recovery of two isozyme variants from progeny of 185 soybean plants regenerated from somatic embryogenesis indicated the feasibility of selection for molecular variants.

2.4.7 Chemical Analysis

Information on the nature and magnitude of genetic variability is of immense value for raising any systematic breeding programs in crops (Simmond, 1962).

Crude protein, cell wall constituents concentrations and dry matter digestibility are some of the important parameters for screening the genetic variability of any forage crop from the live stock nutrition point of view.

The nitrogen content together with the content of cell wall are most important factor to the voluntary consumption (Von Soest, 1994).

Level of the crude protein plays a critical role in meeting the maintenance protein needs of ruminants fed on forage as sole feed for long time as they need protein supplementation to be in positive nitrogen balance when the basal forage falls short of critical protein level of 7% (Wangner, 1989). Among the alternatives to crude fibre to predict energy value of feed stuffs, ADF, NDF and lignin are considered most promising (Pigden *et al.*, 1979 and Krishnamoorthy *et al.*, 1995).

However, CP concentration *per se* is of questionable relevance to the nutrition of ruminants and generally not considered to be a useful selection criterion for improving nutritional value of forages (Smith *et al.*, 1997). Pandey *et al.* (1977) found CP content in the range of 2.8 to 10.5% in *C. ciliaris* across the year between Jan-Feb and June. Similarly for *C. setigerus*, CP was 3.1 and 8.1% in Jan-Feb and June respectively.

Kamadia and Parihar (1988) reported that CP contents varied from 11.7 to 6.3 and 12.9-7.4% in *C. ciliaris* and *C. setigerus* respectively, harvested at 10 and 60 days of interval.

Varaprasad *et al.* (1995) worked on *Cenchrus glaucus* for the nutritional evaluation. On DM basis the average CP, EE, CF, NFE and ash contents of the forage were 7.43, 2.35, 26.41, 53.73 and 10.08 percent respectively.

Krishnamoorthy *et al.* (1996) found that among all the fibrous fractions, ADF had the highest correlation with metabolizable energy (ME), followed ADL, CF, NDF. They defined that the ADF and ADF-ash could be used as substitutes for CF and AIA respectively in quality controls of cattle feeds. Mishra *et al.* (1997) proved that *Cenchrus* based diet can be fed to sheep and goats for maintenance.

Singh and Samanta (1998) demonstrated the chemical composition of *Cenchrus ciliaris*. CP was 3.34%; NDF 78.41%; ADF 51.82%; ADL 9.51% and Ash was found 9.5%.

The nutrient contents including gross energy of three varieties of Anjnan grass (*C. cliliaris*) IGFRI 3108, Mollopo, Marwari anjan, were almost similar and within normal range (Ranjhan, 1993; Kabugo and Darko, 1993 and Reddy *et al.*, 1995b). Pachauri *et al.* (1998) also worked with these three varieties and found that all varieties were comparable nutritionally. CP content varied from 3.91% to 4.76%, CF from 34.85 to 38.41%, NDF from 76.06 to 82.94%, ADF from 52.16 to 52.92% among the varieties. Singh (2001) evaluated five different grasses for nutritional traits. They reported that *C. ciliaris* contained 5.35; 69.90; 42.00; 29.05; 27.90; 6.50 and 89.40% of CP, NDF, ADF, cellulose, hemicellulose, lignin and ON contents, respectively.

There is a wealth of genetic variation for plant traits related to forage nutritional value. Most of these traits could be modified relatively easily by traditional laboratory evaluation and selection methods. Tissue culture technology has a great potential to create novel plant with improved nutritional value. A few major genes have large effects on forage nutritional value trait.

METARIAL AND METHODS

3. MATERIAL AND METHODS

The whole investigation was comprised of two major and sequential groups of studies and accordingly the details of material used and methods applied are described.

First group of studies

Optimization of callus induction and plantlet regeneration from various explants of diverse genotypes of three *Cenchrus* species, viz., *C. ciliaris, C. setigerus* and *C. echinatus*

Second group of studies

Evaluation of somaclones and their progenies, and the comparision with respective parent materials for somaclonal variation.

3.1 Materials

The experimental materials consisted of selected genotypes of three *Cenchrus* species obtained from experimental field of Indian Grassland and Fodder Research Institute, Jhansi. The laboratory investigations were done at the Biotechnology section, Crop Improvement Division, I.G.F.R.I. Jhansi and Plant Animal Relationship Division I.G.F.R.I. Jhansi.

Following genotypes were used as explant source:

Cenchrus cliliaris

1. EC400631
2. EC397600
3. EC400587
4. IG693108
5. EC400610
6. EC397680

Cenchrus echinatus

1. EC397342

Cenchrus setigerus

1. EC400639

3.2 Method

3.2.1 First Group Of Studies

OPTIMIZATION OF CALLUS INDUCTION, CALLUS GROWTH, CALLUS QUALITY AND PLANTLET REGENERATION FROM VARIOUS EXPLANTS OF THE THREE SPECIES OF *CENCHRUS*

(a) Optimization of Callus Induction

Explants

Callus induction was tried from different explants in all the genotypes.

The explants used were:

1. Seeds from field grown plants, after 2-3 months of harvesting.
2. Immature inflorescences which were about to emerge out of the boot leaf.

Sterilization of the Explants

Materials brought from the field were sterilized before inoculation to kill the microbes present on the surface of the explants, otherwise these microbes would contaminate the medium (on which explants are inoculated) and suppress the response of the explants.

Sterilization was done before each inoculation. Different types of explants required different methods of sterilization. All the operations were conducted carefully in a Laminar Air Flow cabinet to ensure aseptic conditions. These operations were as follows:

(1) Mature seed explant

i Seeds were dehusked by kneading.

ii Dehusked seeds were dipped in 70% alcohol for 30 seconds.

iii Then, the seeds were dipped in autoclaved 0.2% Mercuric chloride aqueous solution for 3 minutes with occasional stirring.

iv Subsequently, the seeds were thoroughly washed with autoclaved distilled water for 3-5 times for removing the residue of the sterilizing agent.

v About ten seeds each were put aseptically on the medium which contained in 100ml flask.

(2) Immature inflorescence

i Freshly growing immature inflorescences from well irrigated plants were harvested.

ii After the removal of the boot leaf the inflorescence were dipped in 70% alcohol for 30 seconds.

iii It was followed by dipping in 0.2% autoclaved aqueous solution of Mercuric chloride for 90 seconds.

iv The sterilized inflorescence was washed thoroughly with autoclaved distilled water.

v Inflorescence was cut into smaller pieces of 5mm. length using sterile scalpel blades. Three to four pieces each were placed on the medium in 100ml flask aspectically.

Inoculation

Before inoculation the entire internal surface of the Laminar Air Flow cabinet was wiped with 70% alcohol. The door of the cabinet was tightly closed and the ultra violet germicidal lamp was switched on for 20 minutes for internal sterilization of the cabinet. At the start of inoculation, the UV lamp was switched off and the illuminating tubes were switched on. Now the sterilized explants were placed aspectically on the culture media using sterile forceps.

Media

The following media were used with suitable modifications.

1. Murashige and Skoog medium (MS), formulated by Murashige and Skoog (1962) - Table 3.01
2. SH medium, formulated by Schenk and Hildebrandt (1972) - Table 3.02
3. B_5 medium formulated by Gamborg *et al.* (1968) - Table 3.03

Table 3.01

Composition of Murashige and Skoög (1962) medium and quantity of chenmicals used preparing various stock solutions

Chemical	Concentration (mg/l)	Stock (ml)	Quantity for stock solution	Volume required for one L. medium (ml)
NH_4NO_3	1650	A(500)	16,500	50
KNO_3	1900		19,000	
$MgSO_4.7H_2O$	370		3,700	
KH_2PO_4	170		1,700	
CaCL2.H_2O	440	B(100)	4,400	10
$MnSO_4.4H_2O$	22.3	C(100)	1,115	2
$ZnSO_4.7H_2O$	8.6		430	
H_3BO_3	6.2		310	
KI	0.83		41.5	
$Na_2MoO_4.2H_2O$	0.25		12.5	
$CuSO_4.5H_2O$	0.025		1.25	
$CoCl_2.6H_2O$	0.025		0.125	
$Na_2EDTA.2H_2O$	37.3	D(100)	373	10
$FeSO_4.7H_2O$	27.8		278	
Glycine	2.0	E(100)	200	1
Nicotinic acid	0.5		50	
Pyridoxin.HCl	0.5		50	
Thiamine.HCl	0.1		10	
Myo-inosotol	100	F(100)	1000	10

Sucrose @ 30 g/l

Agar-agar @ 8 g/l

Table 3.02

Composition of Shenk and Hildebrandt (1972) medium and quantity of chemicals used for preparing various stock solutions.

Chemicals	Concentration (mg/l)	Stock (ml)	Quantity for stock solution (mg.)	Volume required one L. medium (ml)
KNO_3	2500	A (500)	25000	50
$MgSO_4.7H_2O$	400		4000	
$NH_4H_2PO_4$	300		3000	
$CaCl_2.2H_2O$	200	B (100)	2000	10
$FeSO_4.7H_2O$	15.0	C (100)	150	10
Na_2EDTA	20.0		200	
$MnSO_4.7H_2O$	10.0	D (100)	100	2
H_3BO_3	5.0		50	
$ZnSO_4.7H_2O$	1.0		50	
KI	1.0		50	
$CuSO_4.5H_2O$	0.2		10	
$NaMoO_4.2H_2O$	0.1		5	
$CaCl_2.6H_2O$	0.1		5	
Inositol	1000	E (100)	10000	10
Thiamine	5.0	F (100)	500	1
Nicotinic acid	5.0		500	
Pyridoxin	0.5		50	

Sucrose @ 30 g/l

Agar-agar @ 8 g/l

Table 3.03

Composition of Gamborg's B5 medium and quantity of chemicals used for preparing various stock solutions.

Chemical	Concentration (mg/l)	Stock (ml)	Quantity for stock solution (mg.)	Volume required for one L. medium (ml.)
NaH_2PO_4	150	A (100)	1500	10
KNO_3	2527.5		25275	
$MgSO_4.7H_2O$	246.5		2465	
$(NH_4)2SO_4$	134.0		1340	
$CaCl_2$	150	B (100)	1500	10
$FeSO_4.7H_2O$	27.8	C (100)	278	10
Na_2EDTA	37.3		373	
KI	0.75	D (100)	37.5	2
H_3BO_3	3.0		150	
$MnSO_4.H_2O$	10.0		500	
$ZnSO_4.7H_2O$	2.0		100	
$NaMoO_4.2H_2O$	0.25		12.5	
$CuSO_4.5H_2O$	0.025		1.25	
$CoCl_2.6H_2O$	0.025		1.25	
Inositol	100	E (100)	1000	10
Thiamine	10.0	F (100)	1000	1
Nicotinic acid	1		100	
Pyridoxin	1		100	

Sucrose @ 30 g/l

Agar-agar @ 8 g/l

The growth regulator in different concentrations and the other adjuvants added in the callus induction media are given below

Media for callus induction

Basal media	Auxin 2, 4-D Mg/l	Cytokinin BAP mg/l	Media Adjuvants Casein hydrolysate mg/l
MS	1.0	0.2	-
	2.0	0.2	-
	3.0	0.2	-
	5.0	0.2	-
	10.0	0.2	-
	3.0	-	-
	3.0	0.5	-
	3.0	0.5	500
SH	3.0	0.2	-
B_5	3.0	0.2	-

In routine, media preparation is time consuming. To avoid this, the stock solutions of the chemicals were used. For preparing stock solutions, the chemicals were dissolved in distilled water in required quantity. The salts were dissolved adding one compound at a time. Dissolving the inorganic nitrogen source (salts) first was usually avoided in all the preparations. All prepared stock solutions were kept in well stoppered and sterilized bottles at 4 - 10^0 C. For preparation of iron stock solution, Na_2EDTA and $FeSO_4.7H_2O$ were dissolved separately with gentle heating then mixed together to make up the final volume. This stock solution was kept in amber coloured bottle for the prevention from light sources.

Media for shoot induction

MS basal media with the following combinations of growth regulators was used as shoot induction media:

Cytokinin mg/l	Auxin mg/l
Kinetin	2,4-D
2.0	0.20
3.0	0.20
5.0	0.20
2.0	0.00
BA	
2.0	0.20
3.0	0.20
5.0	0.20
2.0	0.00

Root inducing media

MS basal media with the following combinations of growth regulators with or without charcoal were used as root induction media:

Auxin IBA (mg/l)	Cytokinin BAP (mg/l)	Activated charcoal (g/l)
0.5	-	-
1.0	-	-
0.5	-	2.0
1.0	-	2.0
2.0	-	2.0
0.5	0.2	-
1.0	0.2	-

Half strength MS medium was also used for this purpose.

Stock solutions of plant growth regulators

Auxins were dissolved in a few drops of alkali and gradually diluted to the required volume with double distilled water. Cytokinis were dissolved in a few drops of dil. HCl heated slightly and gradually diluted to the required volume with double distilled water.

Media adjuvants

Media adjuvants like casein hydrolysate and activated charcoal were added at the time of semisolid media preparation.

Preparation of semisolid media

The stock solutions were mixed in the required proportion in each medium and the growth hormones were added according to requirement. Sucrose was dissolved. Then media adjuvants were added (if needed). The final volume was made up with distilled water. Then, pH was adjusted to 5.6 – 5.8 with the help of 1N HCl or 1N NaOH. Finally agar – agar was added at the rate of 0.8%. Melting of the media was done at 98^0 C for 10 minutes to homogenize the agar – agar in the medium. Melted media was poured into culture vials (100 ml conical flask or culture tubes). Culture vials were plugged with non-adsorbent cotton. Culture vials along with media were autoclaved at 15 lbs per sq. inch pressure for 20 minutes.

Incubation of Culture

For the initiation of callus induction as well as for the maintenance of callus, cultures were kept in dark at 25 ± 2^0C.

Just after shoot induction, the cultures were transferred to light. They were incubated at 25 ± 2^0C and 16 hours light + 8 hours dark photoperiod with 2500 to 3000 lux light intensity.

For root induction, cultures were incubated in same manner as in shoot induction.

Recording of the observation

The observations were recorded on the following aspects

1. **Callus Induction Frequency of Explant :** The number of explants inoculated and the number of explants responded after 20 days of inoculation were counted.

$$\text{Callus induction frequency} = \frac{\text{Number of explant callused}}{\text{Number of explants inoculated}} \times 100$$

2. **Visual Callus Quality Score :** Calli was visually scored 20 days after inoculation on the basis of its regeneration capacity. These scoring is as below-

   0 = Icy white, brown callus with watery texture, non-regenerating type.

   1 = Yellow, fragile callus, non regenerating type.

   2 = Friable, white callus which has little regeneration capacity.

   3 = Milky white or egg white greenish and compact callus, having high regeneration capacity.

Subculturing

Callus was maintained by successive subcultures about every 20days on the same media containing 3.0mg/l 2,4-D and 0.2 mg/l BA, up to five passages. The observation of the callus growth color, texture and other callus quality parameters were recorded.

(b) Optimization of Morphogenesis

Shoot differentiation

The number of shoots formed per 100 mg of calli 20 days after subculture to the regeneration media were recorded.

Root differentiation

The number of roots formed per shoot were recorded.

(c) Histology

The fresh calli of various textures (non regenerating and regenerating) were fixed in Carnoy's solution of acetic acid and alcohol (1:3) for 24 hours and subsequently stored in 70% alcohol. The samples for study were selected and passed through tertairy butyl alcohol dehydration – infilteration series and embedded in petrowax according to Johnsen, 1940.

The sections were cut at 10µ thickness by hand driven micrtome and spread on thoroughly clean microslides. Cleaning of microslides were done by putting them overnight in chromic acid solution and thereafter washing thoroughly

with water and finally with 95% alcohol and dried. Mayer's adhesive (Johnsen, 1940) was used over for affixing the sections onto the slides and a highly diluted Gloi solution was used for floating the adhesive smeared slides to facilited spreading of the sections.

The sections were dewaxed with xylene and brought to water through graded alcohol series. Northen's variations of fosters tannic acid – ferric chloride saffranine and fast green FCF (Johnsen, 1940), stain series was used for staining of sections of calli samples. The sections were cleared with xylene and mounted in DPX mount. The photographs of the sections were taken with upright binocular microscope with automatic exposimeter photomicrographic attachment. (Nikon, Japan).

(d) Hardening of the plantlets

Prior to hardening, the *in vitro* regenerated plantlets were kept on semisolid agar medium containing 0.4% sucrose, prepared with ordinary tap (mineral) water. After 20-21 days of transfer, the plantlets were taken out from the culture vials and washed properly to remove the adhering media. Now these plantlets were placed in test tubes having sterile tap water for 2-3 days in such a way that the roots were always moist. Now they were ready for transfer to the pots. The frequencies of plantlets survived and established 20 days after transplanting were noted.

3.2.2 Second Group Of Studies

EVALUATION OF SOMACLONES AND THEIR PROGENIES, AND THE COMPARISON WITH RESPECTIVE PARENT MATERIALS FOR SOMACLONAL VARIATION

Hardened plantlets were transferred to the field. Seeds were harvested from the somaclones for raising R_1 generation. Somaclones were grown along with their progenies (R_1) and respective parents for field evaluation. The sequence of plants in the field was

Somaclone --------------------progenies----------------------------parent

Plants were planted at the distance of 30 centimeters. After the full growth of plants, somaclones were evaluated for following characters:

(A)Morphological Observations

Data of two seasons for morphological characters were observed.

1. **Qualitative characters**

   The progenies of regenerants (R1), regenerants (R0) and parental genotypes were evaluated for leaf surface, growth habbit and spikelets (bur) color.

2. **Quantitative characters**

   The observations for different forage and seed yield traits were recorded.

(a) **Forage Yield Trait**

- **Days to bloom:** The number of days taken from the date of sowing to the date of each plant when first spike was emerged out.
- **Plant height:** The height was determined by measuring the distance of both the tallest leaf and inflorescence in centimeters from the tips of the best developed tiller to the soil.
- **Tiller Number:** Total number of tillers were counted.
- **Leaf Number:** Total number of leaves per tiller on main tiller were counted.
- **Leaf Length:** The length of leaf blade was measured in centimeters from the base to the tip of blade of the third leaf from the top on the main tiller. Three leaves were selected for this observation. Average leaf length was calculated.
- **Leaf Width:** The breadth of leaf was measured in centimeters at the widest portion of leaf blade of the third leaf from the top of the main tiller. Average leaf width from three leaves were calculated.
- **Internodal Length:** Internodal length between third and fourth node was recorded in centimeters.
- **Green fodder yield:** Plants were harvested individually and immediately weighed separately (in grams) to get the green fodder yield per plant.

- **Dry matter yield:** Harvested plants were air dried, then oven dried at 60^0C for 3 days. Weight (in grams) of each plant was recorded.

(b) **Seed Yield Trait**

- **Peduncle length:** Length of the stalk of spike was measures in centimeters.
- **Spike length:** Length of three spikes were measured in centimeters from the base to the apex of the panicle. Average was calculated.
- **Spike width:** Breadth of three spikes were measured in centimeters at the widest region. Average was calculated.
- **Number of spikelets/spike:** Total number of spikelets present in one spike were counted. Three observations were recorded. Average was calculated.
- **Bur weight:** Weight of 100 burs were recorded in grams.

Six genotypes of *C. ciliaris* and one each of *C. setigerus* and *C. echinatus* were selected for field evaluation. Each plant has a specific number of 6 digits. This number has been used in the experiments of chemical analysis and Biochemical analysis. First number denotes genotype number. Second number denotes explant used. Third and fourth numbers denote the somaclone number, and fifth and sixth numbers denote the progeny number of each somaclone.

There were 8 genotype numbers for all the three species.

For *C. Ciliaris*

Genotype: 1 - EC400631 (100000)

2 - EC397600 (200000)

3 - EC400587 (300000)

4 - IG693108 (400000)

5 - EC400610 (500000)

6 - EC397680 (600000)

For *C. setigerus* genotype number was 7 (700000) and for *C. echinatus* it was 8 (800000). Seed explants were represented by 1 and inflorescence explant was represented by 2.

(B) Biochemical Analysis

Sampling

A total of twenty two somaclones of *C. ciliaris* and one somaclone of *C. setigerus* were sampled for isozyme characterization. The sampling was done based on the analysis of the following characters:

1. Explant
2. Passage
3. Plant height
4. Number of tillers
5. Number of leaves
6. GFY
7. DMY

The selection was done after representing somaclones of each explants and passage. For morphological traits, different groups based on the range of variation were made and each group was represented by a somaclone. In case of *C. setigerus* the only somaclone that exhibited a different mode of reproduction (facultative apomict) from its parent was selected for isozyme analysis. Here also *C. echinatus* could not involve in this experiment because of its annual nature.

Method:

Fresh young leaves of selected somaclones were collected from the field on ice and stored at -20^0C for later use. 300 mg tissue was homogenized by hand in a precooled mortar and with a pestle with the help of 600 ml chilled extraction buffer (100 mM. Tris HCl pH-7.0, containing PVP (m.w. 40,000) = 1.0 %, PVP (m.w 36,000) = 0.5%, Beta – mercaptoethanol 10mM. And sucrose 10%). The extract was centrifuged at 10,000 RPM for 20 minutes. Supernatant was preserved, referred as sample and kept at -20^0C for later use of isozyme analysis and the pellet was discarded. Isozyme in thawed fractions were separated by PAGE (poly acrylamide gel electrophoresis) and bands were detected with the help of a transilluminator. Chemicals used for this purpose are mentioned in Table 3.04.

Discontinuous gel was prepared for each isozyme following Laemmli (1970). It consisted of two gel buffers.

(1) Resolving gel buffer (RGB),

(2) Stacking gel buffer (SGB).

Composition of RGB (20 ml)

Distilled water	-	11.5 ml
RGB (1.5 Trics HCl, pH-8.8)	-	2.5 ml
APS (1.5%)	-	1.0 ml
Acrylamide (30% - 0.8% bis acrylamide)	-	5.0 ml
TEMED	-	0.01 ml

Composition of SGB (10 ml)

Distilled water	-	4.75 ml
SGB (0.5 Tris HCl, pH – 6.8)	-	2.5 ml
Riboflavin (0.6 %)	-	1.25 ml
Acralamide (as in RGB)	-	1.5 ml
TEMED	-	5 µl

Mini protein 11 electrophoretic cell of BIORAD Laboratory was used for gel casting. 20 micro litre of sample was applied into slots, electrophoretic buffer contaqined 3 g/l/ Tris HCl and Glycine 14 g/l. The pH was adjusted to 8.3 by dil. HCl. Electrphoresis was done for 15 minutes at 50 volts, 30 minutes at 75 volts then at 100V until completion of the electrophoretic mobility of samples. The gels were stained, fixed and stored. A total of six isozymes were studied.

(I) ESTERASE

Substrate

i	Alfa naphthyl acetate in 5 ml of 60% acetone	-	60 mg
ii	Sodium di-hydrogen phosphate (0.2 M)	-	20 ml
iii	Di-sodium hydrogen phosphate (0.2 M)	-	10 ml
iv	Distilled water	-	20 ml

Stain

Fast blue RR salt dissolved in 3 ml of acetone - 80 mg

Solution 1, 2, 3, and 4 were mixed to which gels were placed and incubated for 10 minutes, then the stain was added for and distained with 40 ml methanol + 40 ml water + 8 ml ethanol + 4 ml acetic acid.

(II) ACID PHOSPHATASE

Substrate

i 0.33 g. of sodium acetate is weighed and dissolved in distilled water and pH was maintained to 5.5 with acetic acid and made upto 50 ml.

ii 100 mg of alfa naphthyl acid phosphate was dissolved in 5 ml of 70% acetone.

Stain

100 mg Fast Blue RR salt was dissolved in 1 ml of 10% $MgCl_2$. Gel was incubated in solution (i) and (ii) for 10 minutes and stain was added.

(III) GLUCOSE 6 P DEHYDRODENASE (G 6 PDH)

Substrate

i	0.05 M Tris HCl (pH – 8.0)	-	50 ml
ii	Glucose 6 phosphate di sodium salt	-	50 mg

Staining solution in 1 ml of the above buffer

* NADP	-	5 mg
* MTT	-	10 mg
* PMS	-	2 mg
* $MgCl_2$	-	50 mg

Incubated the gel in buffer with substrate and staining solution.

(IV) PHOSPHOGLUCOMUTASE (PGM)

Substrate

i	0.1 M Tris HCl (pH – 7.5)	-	50 ml
ii	Glucose 1 phosphate	-	80 mg
iii	Glucose 6 p dehydrodenase	-	20 units

Staining solution

* NADP	-	10 mg
* MTT	-	15 mg
* PMS	-	1 mg
* ATP	-	15 mg
* $MgCl_2$	-	175 mg

Gel was incubated in buffer with substrate and enzyme for 10 minutes and staining solution was added.

Table 3.04 List of chemicals used for bio chemical analysis

Chemical Name	Molecular Wt.
Acetone	58.08
Acetic acid	60.05
Acrylamide	71.08
Adenosine tri phosphate (ATP)	551.1
Ammonium per sulphate	228.8
Bis acrylamide	154.17
2, 5- diphenyl tetra zolium bromide (MTT)	414.32
Di sodium hydrogen phosphate (Na_2HPO_4)	141.96
Ethanol	46.01
Fast Blue RR salt (4-benzoylamino; 2,5 di methyl benzene diazonium chloride heme $ZnCl_2$ salt)	387.9
Fructose 6 phosphate	304.1
Ethylene diamine tetra acetic acid (EDTA)	372.24
Glucose 1 phosphate (disodium salt)	336.3
Glucose 6 phosphate (disodium salt)	304.1
Glucose 6 P dehydrogenase (1 unit to oxidise 1.0 micro mole of D – glucose 6 phosphate to 6-phospho – D- glucanate per minute in the presence of NADP at pH 7.4 at 25^0C)	
Glycine	75.07
Magnesium Chloride ($MgCl_2$)	203.3
β – mercapto ethanol	78.13
Methanol	32.04
α naphthyl acetate	186.2
α naphthyl acid phosphate	246.3
β – nicotinamide adenine di nucleutide phosphate (NADP)	734.4
Phenazine methosulphate (PMS)	306.3
Poly venyl purrilidone (PVP)	40000
PVP	36000
Riboflavin (Vit. B2)	376.4
Sodium acetate	82.03
Sodium di hydrogen phosphate (NaH_2PO_4)	156.02
Sucrose	342.3
N, N, N`, N` tetra methyl ethylene diamine (TEMED)	116.2
Tris HCL	121.14

(V) **PHOSPHOGLUCOISOMERASE (PGI)**

Substrate

i	0.1 M Tris (pH - 7.5)	-	50 ml
ii	Fructose di phosphate di sodium salt	-	20 mg
iii	Glucose 6 p dehydrogenase	-	20 units

Staining solution

* NADP	-	10 mg
* MTT	-	10 mg
* PMS	-	2 mg
* $MgCl_2$	-	150 mg

Gel was incubated with buffer with substrate for 10 minutes and staining solution was added.

(VI) **SUPER OXIDE DISMUSTASE (SOD)**

i	50mM Tris HCl (pH – 8.0)	-	50 ml
ii	Riboflavin	-	3 mg
iii	EDTA	-	10 mg
iv	MTT	-	10 mg

All solutions were added to the gel. Gel was incubated in dark for 20 minutes then 15 minutes in strong light.

(C) Chemical Analysis (Forage Quality Traits)

Chemical analysis was done in some selected somaclone, progeny and their parent plant samples. Selection was based on some morphological characters like plant height, green fodder yield, dry matter yield, number of tillers, number of leaf and leaf area. Ten somaclones of six genotypes (EC400631, EC397600, IG693108, EC400610, EC397680) of *C. ciliaris* and six somaclones of *C. setigerus* (EC400639) were selected. At the time of harvesting, the selected somaclones with its two progenies and parental genotype drawn for the estimation of dry matter content from the field were processed and subjected to chemical analysis for determination of various quality parameters as described below. *C. echinatus* could not be included in this experiment as senescence just after flowering occurred very fast.

1. **Dry Matter Percentage**

All the parameters were calculated on the basis of dry matter percentage. The known quantity of ground samples of selected plants were taken in already weighed moisture cups and dried in hot air oven at 100^0C for 24 hours. These samples were reweighed after drying to record their respective dry weights. The percent dry matter content was determined by the relationship

$$\text{Dry matter percentage} = \frac{\text{Weight of dry sample}}{\text{Weight of sample taken}} \times 100$$

2. **Crude protein**

The total nitrogen content in the plant samples was determined by micro-kjeldahl method (A.O.A.C., 1975). For this 0.5 g. dried ground samples were transferred in kjeldahl flask and digested in 10 ml of concentrated H_2SO_4 using catalyst mixture (K_2SO_4 + $CuSO_4$ in the ratio of 9:1). The digested material was transferred to 100 ml volumetric flask with distilled water and volume was made upto the mark. An aliquot of 5 ml was distilled with 40% NaOH and the ammonia liberated was absorbed in 5ml of 4% boric acid containing Tashano's mixture indicator (Bromocresol green and methyl red). The distillate was titrated with (N/100) H_2SO_4. Nitrogen was calculated using the expression 1ml of N/100 H_2SO_4 = 0.00014 gram of nitrogen. Crude protein was computed by multiplying the nitrogen percentage with a factor 6.25.

$$\% \text{ C.P.} = \frac{V \times 0.00014 \times D \times 100 \times 6.25}{W \times A}$$

Where,

V = Volume of N/10 H_2SO_4 (Titer value)

D = Dilution factor

W = Weight of sample

A = Aliquot taken

3. **Acid Detergent Fibre (ADF)**

ADF was estimated by the method suggested by Van Soest (1967). One gram ground sample was refluxed with 100 ml of acid detergent solution (cetyl trimethyl

ammonium bromide @ 20g/l, concentrated H_2SO_4 @ 28ml/l) in a 600ml beaker without spout for one hour on a refluxing apparatus. The extracted material was filtered through a tared Gooch crucible under vacuum. Any residue left in the beaker was transferred to the Gooch crucible with hot distilled water (90-100^{0}C) and filtered again. This washing procedure was repeated several time with hot water. Final washing was done with acetone till the filterate was free from colour. The crucible was kept in a dessicator and weight of crucible was expressed as ADF.

$$\% \text{ ADF} = \frac{\text{Weight of crucible + fibre - Weight of sample}}{\text{Weight of sample}} \times 100$$

4. **Acid Detergent Lignin (ADL) and Cellulose**

After ADF extraction, the content of crucible were covered with cooled 72% H_2SO_4 (15^{0}C) and stirred with a glass rod to a smooth paste, breaking all lumps. The crucible was again half filled with 72% H_2SO_4, stirred regularly at an interval of one hour and kept for three hours at 20-23^{0}C. the excess of H_2SO_4 was filtered off under vacuum and content was washed with hot water until free from acid. The crucible were kept in an oven at 100^{0}C for over night, cooled in a dessicator and weighed again. The loss in the weight of crucible was expressed as cellulose content. Crucible were then transferred to muffle furnase and ignited at 550^{0}C for 3 hours. The crucible were cooled in a dessicator and weighed again. The loss in the weight was taken as lignin content.

$$\% \text{ ADL} = \frac{\text{(Weight of crucible + lignin) – (Weight of crucible + ash)}}{\text{Weight of sample}} \text{ X } 100$$

Cellulose = % ADF - % ADL

5. **Neutral Detergent Fibre (NDF)**

Method was followed by estimation of NDF given by Van Soest (1967).

Preparation of the NDF solution:

NDF solution was prepared by dissolving 18.61 g disodium ethylene diamine tetra acetate dihydrate (EDTA) and 6.81 g sodium borate dihydrate in acetate in about

500 ml of distilled water by heating on boiling water bath. Then 30 g of sodium lauryl sulphate dissolved in about 200 ml of hot distilled water was added. To this 4.56 g anhydrous disodium hydrogen phosphate dissolved in 100 ml of hot distilled water, was mixed. After cooling, 10 ml of 2-ethoxy ethanol was added to the mixture and volume was made to one litre.

Procedure:

One gram ground sample was taked in 600 ml spoutless beaker with 100 ml of NDF and heated to boiling for 5-10 minutes (reduce heating as soon as boiling begins, to avoid foaming). Adjusted boiling to an even level and refluxing for 60 minutes after on-set of boiling was done.

Place previously weighed crucible, use vacuum filteration. Rinse sample into crucible with a minimum of hot (80^0C) water. Filter liquid and repeat washing atleast thrice. Wash twice with acetone. Dried crucible at 100^0C for overnight and weighed.

$$\% \text{ NDF} = \frac{(\text{Weight of crucible + NDF}) - (\text{Weight of Crucible})}{\text{Weight of sample}} \text{ X } 100$$

6. **Hemicellulose**

Hemicelluslose was represented by the difference between % NDF and % ADF

% hemicellulose = % NDF - % ADF

7. **Availability index (A.I.)**

A.I. was calculated with the following formulae:

$$\text{A.I.} = 100 - \left[\frac{\text{Lignin x } 100}{\% \text{ NDS (B)}}\right]$$

3.2.3 Statistical Analysis

3.2.3.1 First Group of Study

The statistical methods suggested by Compton (1994) were followed. Analysis of variance for separate effects of media, growth regulators and media adjuvants for callus induction and regeneration with different genotypes were performed using factorial completely randomized design (CRD) with unequal number of replications. Each treatment was replicated at least five times with 2 – 3 explants per replication.

For non parametric traits, Kruskal-Wallis statistic was computed.

Step 1: All of the observations in k groups were ranked in a single series assuming ranks from 1 to N tied observations were assigned the value of the average of the tied ranks.

Step 2: The Kruskal-Wallis statistic was calculated as follows-

$$KW = \frac{12}{N(N+1)} \sum_{j=1}^{k} nj \; (Rj-R)^2$$

Where

k = number of samples or groups

nj = number of cases in the j^{th} group

N = number of cases in the combined group

Rj = average of the ranks in the j^{th} group

R = (N+1)/2 = the average of ranks in the combined sample (the grand mean) and the summation is across k samples.

3.2.3.2 Second Group of Study

The basic descriptive statistics consisting of range, mean, standard deviation and coefficient of variation were computed. The variance in quantitative characters were determined statistically as proposed by Fukui (1986). A variant is defined as the plant with atleast one trait falling outside the limits set by the following formula

$M \pm \delta \sqrt{[F(N+1)/N]}$

Where,

M = the mean value of the trait of the control

δ = the standard deviation of the trait of the control

N = is number of plants in the control

F = the table F value (at $\alpha = 0.05$ n 1 = 1, n2 = N-1)

This criteria was applied to estimate the number of variants for each of the quantitative character under study.

The frequency distribution of various quantitative traits were worked out for each genotype and plotted using the class mid – points on the X - axis and the number of plants (frequency) on Y – axis.

Isozyme:

The band pattern of isozyme in many somaclones were selected for studying somaclonal variation. The zymograms were recorded and the isozyme bands were scored as present (as 1) or absent (as 0) at every loci to derive the electrophoretic phenotype (EP) for each sample. A matrix of simple matching coefficient was generated using the zymogram data and a phenogram was generated with the unweighed pair-group method using an arithmetic average (UPGMA) according to Sneath and Sokal (1973).

EXPERIMENTAL RESULTS

4. Experimental Results

The results of the present investigation are presented below under two major groups of experiments.

4.1 First Major Group: Optimization of callus induction, callus quality, callus growth and plantlet regeneration from various explants in different genotypes of the three *Cenhrus* species.

4.2 Second Major Group: Evaluation of somaclones and their progenies and the comparision with respective parent material for somaclonal variation.

4.1 Optimization of callus induction, callus quality, callus growth and plantlet regeneration from various explants in different genotypes of the three *cenchrus* species

4.1.1 Callus Induction

4.1.1.1 Effect of Media

(A) Frequency

(i) Seed Explant:

The effect of different media (B_5, MS and SH) for callus induction frequency from seed explant of six genotypes of *C.ciliaris* (EC397680, EC400631, EC397600, IG693108, EC400587, EC400610), *C.setigerus* (EC400639) and *C.echinatus* (EC397342) and their interaction are presented in table 4.01.

Seed explant of six genotypes of *C.ciliaris* and one each of *C.setigerus* and *C.echinatus* exhibited highly significant differences for callus induction frequency on different basal media used. MS medium gave best response (81.93%) for callus induction followed by SH medium (48.19%). The minimum callus induction response was observed on B_5 medium.

There was no significant difference among the genotypes of different species of *Cenchrus* for callus induction response. In term of callus induction frequency, *C.echinatus* (EC397342) was best (64.25%) (Plate # 3, fig.2) which was at par with IG693108 (62.95%) (Plate # 2 fig.1) and EC400610 (60.00%) of *C.ciliaris*. An average callus induction frequencies of rest of the genotypes of *C.ciliaris* and *C.setigerus* were more than 50%.

There was a significant difference for callus induction frequency for interaction between genotypes and media. The maximum callus induction frequency was observed in EC397342 of *C.echinatus* (100%) on MS medium while the same genotype exhibited minimum callus induction frequency on SH medium (17.76%).

(ii) Immature inflorescence explant

The response of different basal media (B_5, MS and SH) for callus induction frequency from immature inflorescence explant of the six genotypes of *C.ciliaris* (EC397680, EC400631, EC397600, IG693108, EC400587, EC400610), *C.setigerus* (EC400639) and *C.echinatus* (EC342347) and their interation are presented in table 4.02.

As revealed by callus induction frequency, there was highly significant difference among media for various genotypes of *Cenchrus* under study. MS medium was found better (60.14%) compared to SH (49.90%) and B_5 (34.34%) media.

Highly significant difference was also observed among different genotypes of *Cenchrus* species. Average callus induction frequency was found best in *C.setigerus* (EC400639) (79.24%) (Plate # 5 Fig.1). Highly responding genotype of *C.ciliaris* for callus induction was EC400587 (66.84%) (Plate # 5 Fig. 1). Genotypes EC400631 (56.65%), EC400610 (53.67%) and EC397600 (53.32%) of *C.ciliaris* were almost equal in performance. Genotype IG693108 of *C.ciliaris* showed the poorest callus induction response (5.55%) from inflorescence explant.

Interaction between genotypes and media were not significant. Callus induction ranged between 91.66% on MS in *C.setigerus* to 0.00% in IG693108 of *C.ciliaris* on SH and B_5 media. Inflorescence explant of IG693108 of *C.ciliaris* reported for callus induction only on MS medium, while the inflorescence explants of *C.cehinatus* did not show any callus induction response on any of the culture media used.

(B) Quality

(a) *C.ciliaris*

(i) Seed explant:

The calli from seed explant of six genotypes of *C.ciliaris* were used to study the effect of different basal media (MS, SH and B_5) on callus colour and texture. The effect is presented in the table-4.03.

There was no significant difference among all the six genotypes for colour and texture of calli. However, the media exhibited significant differences among them for both the characters.

With respect to callus colour, for seed explant, MS medium performed better on a visual score of 1.56 followed by the SH medium (1.11). B_5 medium (0.76) proved to be the poorest for the callus colour and differed significantly from MS medium. Mean value showed that the genotype EC397680 (1.52) scored best among all genotypes followed by the EC400610 (1.22). The genotypes EC400587 (1.14) and EC397600 (1.13) were almost similar for callus colour. The poorest score for callus colour was recorded in the genotype EC400631 (0.84) followed by the genotyope IG693108 (1.01).

For callus texture also, MS medium was found to be the best (1.57) followed by SH medium (0.97). B_5 medium performed comparatively poor (0.88) among all the three media for texture of calli. The best genotype for callus texture was EC397680 (1.56) as revealed by the mean scores. Genotypes EC397600 (1.21) and IG693108 (1.18) were comparable to each other followed by the genotype EC400610 (1.11). Least score for callus texture (vitrious) was noted in the genotype EC400631 (0.83) and EC 400587 (0.94).

(ii) Immature inflorescence explant:

Effect of different basal media (MS, SH and B_5) on colour and texture from immature inflorescence derived callus of six genotypes of *C.ciliaris* is presented in table 4.04.

There was significant difference among the genotypes as well as among the media for callus colour and texture. For callus colour, MS media was found the best (1.78) among all the media followed by SH medium (1.12). B_5 medium showed significant difference from the MS medium and exhibited poorest

Table 4.01: Effect of different basal media on callus induction frequency from seed explant

Genotypes	Different basal media with 3.0 mg/l. 2,4-D			
C,ciliaris	B5	MS	SH	Mean
EC397680	41.60	76.64	58.33	58.88
EC400631	31.66	85.00	55.00	57.22
EC397600	41.00	75.00	45.00	53.7
IG693108	30.53	91.66	66.66	62.95
EC400587	56.66	62.53	39.33	53.87
EC400610	46.66	83.33	50.00	60.00
C.setigerus EC400639	22.20	78.33	53.33	51.28
C.ehinatus EC397342	75.00	100.00	17.76	64.25
Mean	43.18b	81.93a	48.19b	

F Test

Genotype	0.52
Media	29.24**
Interaction	2.24*

Table 4.02: Effect of different basal media on callus induction frequency from immature inflorescence explant

Genotypes	Different basal media with 3.0 mg/l. 2,4-D			
C.ciliaris	B5	MS	SH	Mean
EC397680	8.33	37.20	19.43	21.65 c
EC400631	27.77	75.00	67.20	56.65 ab
EC397600	37.20	61.66	61.10	53.32 b
IG693108	0.00	16.66	0.00	5.55 c
EC400587	61.66	72.20	66.66	66.84 ab
EC400610	44.40	66.64	49.96	53.67 ab
C.setigerus EC400639	61.06	91.66	85.00	79.24 a
Mean	34.34b	60.14a	49.90ab	

F test
Genotype 18.58**
Media 11.08**
Interaction 0.59

Table 4.03: Effect of different basal media on colour and texture of callus induced from seed explants of *C.ciliaris* genotypes

Genotype	Basal media with 3.0 mg/l 2,4-D + 0.2 mg/l BA							
C.ciliaris	MS		SH		B_5		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	1.16	1.21	0.72	0.72	0.66	0.58	0.84	0.83
EC400587	1.50	1.16	1.33	0.85	0.60	0.83	1.14	0.94
EC397600	1.49	2.00	1.12	0.91	0.79	0.72	1.13	1.21
EC400610	1.50	1.33	1.16	1.00	1.00	1.00	1.22	1.11
EC397680	1.90	1.73	1.52	1.52	1.16	1.45	1.52	1.56
IG693108	1.83	2.00	0.83	0.83	0.38	0.73	1.01	1.18
Mean	1.56	1.57	1.11	0.97	0.76	0.88		

	Genotype		Media	
	Color	Texture	Color	Texture
KW	9.32	8.11	10.77	12.65
Probability	0.09	0.15	0.00	0.00

Table 4.04: Effect of different basal media on colour and texture of callus induced from inflorescense explants of *C.ciliaris* genotypes

Genotype	Basal media with 3.0 mg/l 2,4-D + 0.2 mg/l BA							
C.ciliaris	MS		SH		B5		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	2.61	2.38	1.50	1.75	1.16	1.33	1.60	1.82
EC400587	1.93	1.44	1.32	1.26	1.19	1.09	1.48	1.26
EC397600	1.66	1.13	1.23	1.38	1.20	1.18	1.36	1.23
EC400610	1.41	1.45	1.31	1.16	1.25	1.16	1.32	1.25
EC397680	1.51	1.47	1.39	1.50	1.30	1.21	1.4	1.39
IG693108	1.58	1.36	0.00	0.00	0.00	0.00	0.52	0.45
Mean	1.78	1.53	1.12	1.17	1.01	0.99		

	Genotype		Media	
	Color	Texture	Color	Texture
KW	13.27	17.70	17.06	20.36
Probability	0.02	0.00	0.00	0.00

callus colour (1.01). Genotype exhibited significant difference in colour. The highest performing genotype was EC400631 with 1.60 colour score, which was at par with EC400587 (1.48) followed by EC397680 (1.40). The lowest performance was observed in the genotype IG693108 (0.52) followed by EC400610 (1.32) and EC397600 (1.36).

With respect to callus texture, MS medium was observed to be the best with 1.53 visual score followed by the SH medium (1.17). B_5 medium was poorest (0.99) among all and differed significantly form MS medium. Among all genotypes tried, EC400631 exhibited the best response (1.82) for callus texture followed by EC397680 (1.39) and EC400587 (1.26). The genotype EC397610 and EC397600 with 1.25 and 1.23 score for callus texture were significantly different from EC400631. Minimum score for texture was recorded in the genotype IG693108 (0.45).

(b) *Cenchrus setigerus:*

Different basal media (MS, SH and B_5) were used to study their effect on colour and texture of callus induced from the seed and immature inflorescence explants of *C.setigerus*. The data analyzed by KW test is presented in table 4.05.

There was no significant difference among media for colour as well as the texture of the callus from both of the explant. In case of seed explant MS media proved to be most suited for colour and texture followed by the SH media. B_5 media yielded the poorest type of callus for both the characters.

In case of inflorescence explant, MS media was found best only for callus colour where as SH medium was found best for callus texture. B_5 responded moderately.

(c) *C.echinatus:*

The effect of different basal media and varying concentrations of 2,4-D and BA on the colour and texture of callus induced from the seed explants of *C.echinatus* is presented in table 4.06.

All the three basal media (MS, SH and B_5) did not exhibit significant difference among them. MS medium scored maximum for both the characters

(callus colour and texture) followed by SH medium where as B_5 medium had minimum score for these characters.

MS basal media was used to study the effect of different levels of BA and 2,4-D on callus colour and texture.

The effect of BA was not significant for colour and texture. All the combinations exhibited almost similar quality (colour and texture) except the media devoid of BA.

The varying concentrations of 2,4-D also did not effect significantly on callus colour and texture, both. All the concentration of 2,4-D were comparable except the media containing 10.0 mg/l 2,4-D which showed poorest quality of callus.

4.1.1.2. Effect of 2,4-D

(A) Frequency

(a) *C.ciliaris:*

(i) Seed explant

Callus induction frequency (expressed as per cent respond 20 days after inoculation) from seed explant in six genotypes of *C.ciliaris* significantly differed from one another with varying levels of 2,4-D on MS basal media containing 0.2 mg/l BA (table 4.07)

The best average response was seen in the genotype EC400610 (73.9%). The genotype EC397680 recorded a callus induction frequency of 65.5 per cent which was at par with EC400610. The genotype EC397600 and EC400631 were also comparable to EC397680 (Plate # 3 Fig.1). Among all genotypes the performance of IG693108 was lowest in callus induction frequency (13.5%).

Across all treatments, the 2,4-D concentration of 5 mg/l in culture medium excelled in callus frequency (62.5%) which was at par with 3 mg/l of 2,4-D (61.8%) followed by 2 mg/l (56.9%) and 1 mg/l 2,4-D (50.9%) as indicated by per cent explant response for callus induction. When concentration of 2,4-D was increased beyond 5 mg/l, the response came down (45.4%) at 10 mg/l of 2,4-D.

Table 4.05: Effect of different basal media on colour and texture of callus induced from different explants of *C.setigerus*

Media (with 3.0 mg/l 2,4-D + 0.2 mg/l BA)	Explant			
	Seed		Inflorescence	
	Colour	Texture	Colour	Texture
MS	1.83	0.93	1.44	1.18
SH	1.44	0.58	1.16	1.43
B5	0.91	0.66	1.35	1.33
KW	2.85	3.40	.84	1.48
probability	0.24	0.18	0.65	0.47

Table 4.06: Effect of different basal media and varying levels of 2,4-D and BA on colour and texture of callus from seed explant of *C.echinatus*

Media	Explant Seed	
	Colour	Texture
MS	2.08	2.60
SH	1.71	2.28
B5	1.18	0.80
KW	2.99	5.54
probability	0.22	0.62

BA Concentrations	Explant Seed	
	Colour	Texture
0.00	1.75	
0.20	2.85	2.25
0.50	2.67	2.37
0.50+500 mg/l CH	2.62	1.9
KW	5.44	5.35
probability	0.14	0.14

2,4-D Concentrations	Explant Seed	
	Colour	Texture
1.00	1.50	1.50
2.00	1.25	0.87
3.00	1.75	1.37
5.00	1.75	1.36
10.00	0.62	0.57
KW	6.37	5.81
probability	0.17	0.21

Table 4.07: Effect of 2,4-D on callus induction frequency (%) from seed explant of six genotypes of *C.ciliaris*

S. No.	Genotype	2,4-D concentration (with BA 0.2 mg/l in MS medium)					
		1.00	2.00	3.00	5.00	10.0	
1	EC400631	57.6	70.1	54.5	60.2	46.2	56.9bc
2	EC400587	50.0	72.0	51.4	51.8	44.2	52.5 cd
3	EC397600	45.2	66.1	71.1	64.8	55.8	60.7 bc
4	EC400610	59.6	67.7	85.8	86.1	69.4	73.9 a
5	EC397680	72.8	33.2	93.4	73.9	50.0	65.5 ab
6	IG693108	5.0	0.0	26.7	18.1	15.8	13.3 e
Mean		50.9 bc	58.9 ab	61.8 a	62.5 a	45.4 c	

F Test
Genotype 23.52**
2,4-D 4.66**
Genotype 2,4-D 1.50

Table 4.08: Effect of 2,4-D on callus induction frequency of six genotypes of *C.ciliaris* from immature inflorescence explant

Genotype	2,4-D concentration with BA (0.2 mg/l) in MS medium					
	1.00	2.00	3.00	5.00	10.00	Mean
EC400631	31.11	45.53	87.77	46.66	21.66	46.55 a
EC400587	19.43	17.76	27.76	35.00	8.33	21.66 b
IG693108	16.66	28.86	35.00	21.66	0.00	20.44 bc
EC397680	15.00	10.00	19.43	15.00	11.10	14.10 c
EC400610	8.33	27.76	49.96	23.33	25.00	26.88 ab
EC397600	5.00	44.40	55.53	39.96	33.30	44.64 a
Mean	23.42 bc	29.05 b	45.91 a	30.27 b	16.56 c	

F test
Interaction 0.64
Genotype 5.61**
2,4-D: 4.39**

The interaction of different genotypes and varying 2,4-D level was not significant. The maximum response (93.4%) was from genotype EC397680 at 3 mg/l 2,4-D. However, genotype IG693108 exhibited the minimum response (5.0%) at 1 mg/l 2,4-D and no response for callus induction at 2.0 mg/l 2,4-D.

(ii) Immature inflorescence explant

Callus induction frequency (expressed as per cent respond 20 days after inoculation) from immature inflorescence explant in six genotypes of *C.ciliaris* significantly differed from one another with varying levels of 2,4-D on MS basal media containing 0.2 mg/l BA (table 4.08).

The callus induction frequency differed significantly from one another due to differing genotypes. The difference was highly significant among genotypes. Maximum average callus induction frequency was noticed in the genotyoe EC400631 (46.55%) that was at par with EC397600 (44.64%) followed by EC400610 (26.88%). Genotype EC400587 and IG693108 were comparable to each other with 21.66% and 20.44% callus induction frequency, respectively. EC397680 exhibited minimum callus induction response (14.10%).

The difference of varying level of 2,4-D was highly significant. Highest callus induction response was observed with 3.0 mg/l (45.91%). No other concentration of 2,4-D was comparable with 3.0 mg/l 2,4-D. 2,4-D with 5.0 mg/l 2,4-D showed average callus induction frequency (30.27%) which was at par with 2.0 mg/l 2,4-D (29.05%) followed by 1.0 mg/l 2,4-D (23.42%). Minimum callus induction response was noticed with 10.0 mg/l 2,4-D (16.56%).

Interaction between genotype and different 2,4-D concentration was not significant. Callus induction frequency varied between 87.77 % in genotype EC400631 with 3.0 mg/l 2,4-D to 0.00% in genotype IG693108 with 10.0 mg/l 2,4-D and 5.0% in the genotype EC397600 with 1.0 mg/l 2,4-D.

(b) *C.setigerus:*

The effect of different concentrations of 2,4-D was not significant for callus induction frequency (expressed as per cent respond 20 days after inoculation) in seed explant of *C.setigerus* (EC400639) (table 4.9). Maximum callus induction was observed with 5.0 mg/l 2,4-D (35.0%) followed by 3.0 mg/l 2,4-

D (26.38%). Average callus induction frequency noted was 20.69%. The data indicated that the callus induction frequency on the culture media contained lower (1.0 mg/l) or higher (10.0 mg/l) concentration of 2,4-D. In case of immature inflorescence explant the data in callus induction response per explant after 20 days of inoculation on the MS media having different concentrations of 2,4-D and 0.2 mg/l BA were recorded. Media did not affect significantly in *C.setigerus*. The average callus induction frequency was 17.17%. This genotype showed highest performance for callus induction with 2.0 mg/l 2,4-D (33.33%) followed by 3.0 mg/l 2,4-D (22.0%). The performance for callus induction was markedly poor (8.33%) on the culture media containing lower concentration of 2,4-D (1.0 mg/l). the callus induction frequency was also reduced to 11.11% in the culture media containing higher concentration of 2,4-D (5.0 or 10.0 mg/l).

(c) *C.echinatus*

The data in the callus induction response per explant after 20 days of innoculation of the seed of *C.echinatus* on the medium containing different concentrations of 2,4-D were recorded and this has been presented in table 4.10 (Plate # 3, fig. 2)

The effect of 2,4-D was not significant for callus induction frequency. Average callus initiation response was 48.49%. The highest response for callus induction was found to be 56.25% with 5.00 mg/l 2,4-D and the lowest response with 10.00 mg/l 2,4-D (5.99%). The callus induction response on the culture media containing 2.0 mg/l or 3.0 mg/l 2,4-D were at par, 44.57% and 44.44% respectively. The response of 1.0 mg/l 2,4-D containing culture medium was also exhibited to be moderate (37.27%).

(B) Quality

(a) *C.ciliaris*

(i) Seed explant

MS basal medium supplemented with 0.2 mg/l BA was used to study the effect of different levels of 2,4-D on callus colour and texture induced from seed explants of six genotypes of *C.ciliaris*. The data are presented in table – 4.11.

The varying conacnetration of 2,4-D showed significant difference among them for callus colour as well as the texture. Best quality of callus in terms of colour and texture was observed on the medium containing 3.0 mg/l 2,4-D followed by the medium containing 5.0 mg/l 2,4-D. Media containing1.0 and 10.0 mg/l 2,4-D performed comparatively poor for callus quality (colour and texture). The medium having 2.0 mg/l 2,4-D was moderate in performance.

Genotypes did not affect significantly on callus colour and texture. Callus colour was found best in the genotype EC397600 (1.27), EC400631 (1.20) and IG693108 (1.17) with yellowish colour (Plate # 6, fig.2). Genotype EC397680 (0.71) and EC400587 (0.99) were found similar with brownish yellow colour of callus (plate # 6 fig. 1). With regard to callus texture all genotypes were almost comparable producing friable to some what granular type of callus (Plat # 7 fig.1) except EC400631 which exhibited only friable type of callus.

(ii) Immature inflorescence explant

Effect of different concentration of 2,4-D on colour and texture of the callus induced from immature inflorescence explant of six genotypes of *C.ciliaris* is presented in table - 4.12.

MS medium supplemented with 0.2 mg/l BA was used to study the effect of different levels of 2.4-D KW analysis for colour and texture revealed that there was a significant difference among genotypes of colour. Genotype EC400631 showed (1.72) best response and significantly differed from the genotypes EC400610, IG693108 and EC400587, which was followed by the genotype EC397680 (1.26) and EC397600 (1.08). Least response was observed by the genotype EC400587 (0.43) followed by IG693108 (0.88) and EC400610 (0.91). With regard to texture, the genotypes did not exhibit significant difference among them however, mean value of the genotype EC400631 (2.17) ranked on top with greenish colour and granular texture (Plate # 7 fig. 2). Genotype EC400610 (1.57), EC397680 (1.56) and IG693108 (1.48) were comparable having friable to granular texture. EC400587 (1.23) and EC397600 (1.39) responded poorly in terms of texture of callus.

There was significant difference among media for colour and texture of callus. For callus colour media containing 3.0 mg/l 2,4-D was best (1.62) with

Table 4.09: Effect of 2,4-D on callus induction frequency of *C.setigerus*

Basal media	Callus induction frequency (%)	
MS	EXPLANT	
2,4-D concentrations with 0.2 mg/l BA	Seed	Immature inflorescence
1.00 mg/l	15.00	8.33
2.00 mg/l	26.11	33.33
3.00 mg/l	26.38	22.00
5.00 mg/l	35.00	11.11
10.00 mg/l	20.00	11.11
Mean	20.69	17.17
F test	20.69	0.46

Table 4.10: Effect of 2,4-D on callus induction frequency from seed explants of *C.echinatus*

2,4-D concentrations (with 0.2 mg/l BA in MS medium)	Callus induction frequency (%)
1.00 mg/l	37.27
2.00 mg/l	44.57
3.00 mg/l	44.44
5.00 mg/l	56.25
10.00 mg/l	5.99
Mean	48.49
F test	0.48

Table 4.11: Effect of different concentrations of 2,4-D on colour and texture of callus induced from seed explants of *C.ciliaris*

Genotype	2,4-D concentrations (with 0.2 mg/l BA in MS medium)											
c.ciliaris	1.0		2.0		3.0		5.0		10.0		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	1.25	1.00	1.10	1.10	1.50	1.25	1.16	1.16	1.00	0.97	1.20	1.09
EC400587	0.62	1.25	0.95	1.45	1.62	1.97	1.37	1.94	0.41	0.87	0.99	1.50
EC397600	0.60	1.87	0.88	1.02	2.25	1.87	1.62	1.85	1.02	0.79	1.27	1.48
EC400610	0.50	0.75	0.62	1.16	2.08	2.22	1.49	2.12	0.64	0.84	1.06	1.42
EC397680	0.32	0.65	0.62	1.34	1.16	1.60	1.0	1.75	0.49	1.00	0.71	1.27
IG693108	0.76	0.87	1.29	1.15	1.62	2.25	1.32	2.25	0.90	0.98	1.17	1.50
mean	0.67	1.06	0.91	1.20	1.70	1.86	1.32	1.84	0.74	0.90		

	Genotype		Media	
	Colour	Texture	Colour	Texture
KW	9.44	2.33	33.65	40.07
Probability	0.09	0.80	0.00	0.00

Table 4.12: Effect of different concentrations of 2,4-D on colour and texture of callus induced from immature inflorescence explants of *C.ciliaris*

Genotype	2,4-D concentrations (mg/l) with 0.2 mg/l BA in MS medium											
c.ciliaris	1.0		2.0		3.0		5.0		10.0		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	1.50	1.75	1.75	1.87	2.50	3.00	2.00	2.87	0.87	1.37	1.72	2.17
EC400587	0.10	1.02	0.15	0.90	1.00	1.62	0.80	1.60	0.10	1.04	0.43	1.23
EC397600	1.16	1.07	0.65	1.37	1.75	1.94	1.00	1.97	0.87	0.62	1.08	1.39
EC400610	0.39	1.00	0.72	1.29	1.37	2.45	1.27	2.25	0.82	0.87	0.91	1.57
EC397680	0.51	0.87	1.17	1.85	1.80	2.25	1.82	1.85	1.00	1.02	1.26	1.56
IG693108	0.65	0.64	0.62	1.75	1.33	1.80	1.19	2.00	0.62	1.25	0.88	1.48
mean	0.71	1.05	0.84	1.50	1.62	2.17	1.34	2.09	0.71	1.02		

	Genotype		Media	
	Colour	Texture	Colour	Texture
KW	20.61	6.77	19.33	36.14
Probability	0.00	0.23	0.00	0.00

Table 4.13: Effect of varying levels of 2,4-D on colour and texture of callus induced from different explants of *C.setigerus*

Concentrations of 2,4-D (mg/l) with 0.2 mg/l BA in MS medium	Explant			
	Seed		Inflorescence	
	Colour	Texture	Colour	Texture
1.00	0.61	0.82	0.51	0.82
2.00	0.97	1.00	0.62	1.25
3.00	2.00	2.00	2.00	2.75
5.00	1.87	2.20	1.75	2.70
10.00	0.70	0.87	0.75	1.30
KW	8.39	7.76	5.29	8.06
Probability	0.07	0.10	0.25	0.09

yellowish green colour followed by the media 5.0 mg/l 2,4-D. These two media significantly differed from the media containing 1.0 mg/l 2,4-D and 10.0 mg/l 2,4-D which showed brownish yellow colour of callus (0.71 each), followed by the media containing 2.0 mg/l 2,4-D (0.84). The texture was found the best with media containing 3.0 mg/l (2.17) which was at par with media containing 5.0 mg/l 2,4-D (2.09) exhibiting granular type of callus followed by the supplemented with 2.0 mg/l 2,4-D (1.50). Media containing 1.0 mg/l 2,4-D and 10.0 mg/l 2,4-D showed friable callus with minimum scores of 1.05 and 1.02, respectively.

(b) *C.setigerus:*

MS basal medium supplemented with 0.2 mg/l BA was used to study the effect of varying levels of 2,4-D for callus colour and texture of *C.setigerus* (table – 4.13)

The media with different concentrations of 2,4-D did not differ significantly for both the explants. In seed explant callus colour was found best with the media containing 3.0 mg/l 2,4-D while callus texture was scored maximum on the media containing 5.0 mg/l 2,4-D followed by the media 3.0 mg/l 2,4-D. Rest of the media performed almost poorly. In case of immature inflorescence explant, media containing 3.0 mg/l 2,4-D was found more suitable for callus colour and texture followed by the media containing 5.0 mg/l 2,4-D. Media supplemented with 10.0 and 2.0 mg/l 2,4-D were comparable. Least response was observed in the media containg 1.0 mg/l 2,4-D.

(c) *C.echinatus:*

The effect of varying levels of 2,4-D on colour and texture of calli are described in clause 4.1.1.1 Bc and table – 4.06

4.1.1.3 Effect of BA

(A) Frequency

(a) *C.ciliaris:*

(i) Seed explant

The seed of six genotypes of buffel grass were used as explant source at different levels of BA concentration with MS media containing 3.0 mg/l 2,4-D

and the per cent callus induction response after 20 days of inoculation was recorded (table – 4.14).

There was highly significant difference among genotypes. EC397600 was the best genotype (73.7%) followed by EC600610 (61.4%) and EC400631 (60.2%) for callus induction (Plate # 2, fig. 2 and Plate # 3, fig. 2). Callus induction frequency was recorded lowest in IG693108 (21.2%). However, EC400587 (Plate # 3, fig. 1) was better than IG693108 with 40.7 per cent of callus induction frequency.

Highly significant difference was found among varying BA levels. BA with 0.5 mg/l elicited best response (61.0%) for callus induction frequency which was similar to 0.20 mg/l (60.9%). In absence of BA, callus induction frequency was markedly affected (40.1%). BA (0.5 mg/l) in combination with CH (500 mg/l) recorded moderate callus induction frequency.

Interaction between genotypes and different BA levels showed highly significant differences. Callus induction frequency varied between 93.4 per cent in EC397680 at 0.2 mg/l BA to 0.00% in IG693108 in absence of BA. In absence of BA, out of six genotypes, IG693108 exhibited no callus induction at all, EC400587 showed very poor callus induction frequency (11.1%), EC397680 showed maximum callus induction frequency (63.3%) and rest of the genotypes ranged between 47.5% to 53.9% for callus induction frequency which were almost at par. Among all genotypes IG693108 performed lowest in all cases. Addition of casein hydrosylate did not influence much the callus induction frequency.

(ii) Immature inflorescence explant

Immature inflorescence segments of the same six genotypes of C.ciliaris were used as explant source at different levels of BA concentrations with MS medium containing 3.0 mg/l 2,4-D and the data in the terms of callus induction response per explant after 20 days of inoculation of the immature inflorescence on the medium were recorded (Table 4.15).

Highly significant difference was observed among genotypes but different level of the BA did not exhibit significant difference. Genotype EC400631 with highest callus induction frequency (57.5%) was at par with EC397680 (51.8%)

Table 4.14: Effect of BA on callus induction frequency (%) from seed explants of six genotypes of *C.ciliaris*

S.No.	Genotype	BA concentrations (mg/l) with 2,4-D 3 mg/l in MS medium				Mean
		0.0	0.2	0.5	0.5+500mg/l CH	
1	EC400631	47.5	54.5	69.8	76.1	60.2 bc
2	EC400587	11.1	51.4	48.8	38.9	40.7 d
3	EC397600	52.3	71.2	78.3	77.7	73.7 a
4	EC400610	53.9	87.2	49.7	61.2	61.4 bc
5	EC397680	63.3	93.4	60.0	70.0	70.7 ab
6	IG693108	0.0	26.7	36.7	22.2	21.2 e
Mean		40.1 c	60.9 a	61.0 a	53.7 ab	

F test	
Genotype	26.50**
BA	9.07**
Genotype x BA	2.13**

Table 4.15: Effect of BA on callus induction frequency (%) from immature inflorescence of six genotypes of *C.ciliaris*

S.No.	Genotype	BA concentrations (mg/l) with 2,4-D 3.0 mg/l in MS medium				Mean
		0.0	0.2	0.5	0.5+500mg/l CH	
1	IG693108	50.5	22.0	50.5	99.3	51.8 ab
2	EC400587	36.4	17.5	33.8	60.0	42.7 abc
3	EC397600	58.3	46.7	58.3	49.1	51.4 ab
4	EC400610	33.8	37.8	29.2	33.7	34.3 bc
5	EC400631	0.8	50.0	0.8	13.0	11.1 d
6	EC397680	55.6	37.5	66.7	66.7	57.5 a
Mean		37.0	36.1	32.1	53.8	

F test	
Genotype	3.47**
BA	1.74
Genotype x BA	0.76

Table 4.16: Effect of different concentrations of BA on callus induction frequency of *C.setgerus*

Basic media	Callus induction frequency	
MS	EXPLANT	
BA concentrations (mg/l) with 3.0 mg/l 2,4-D	Seed	Immature inflorescence
0.00	59.02	23.33
0.20	26.38	22.22
0.50	58.52	64.28
0.50+500 mg/l CH	53.54	33.33
Mean	49.36	35.79
F test	2.37	0.92

Table 4.17: Effect of BA on callus induction frequency from seed explants of *C.setigerus*

BA concentrations (mg/l) with 3.0 mg/l 2,4-D	Callus induction frequency (%)
0.00	46.2
0.20	44.44
0.50	30.87
0.50+500 mg/l CH	49.62
Mean	42.53
F test	0.85

and EC397600 (51.4%) followed by EC400587 (42.7%). The genotype IG693108 recorded minimum callus induction frequency of 11.1 per cent. EC400610 was moderate among all genotypes in terms of callus induction frequency (34.3%).

With regard to different concentrations of BA, no significant difference was found. BA 0.5 mg/l with 500 mg/l CH in the culture medium elicited best response (53.8%), followed by the absence of BA (37.0%) callus induction frequency decreased with increase in BA level with least response of 32.1 per cent callus induction at 0.5 mg/l BA. Major enhancement in callus induction frequency with the addition of CH was observed.

Interaction between genotypes and media was not significant. The highest callus induction was represented by the genotype EC397680 (99.3%) with 0.5 mg/l BA + 500 mg/l CH and the minimum callus induction response (0.8%) was observed in the genotype IG693108 with 0.5 mg/l BA in the culture medium.

(b) *C.setigerus:*

The callus induction frequency (expressed as per cent response after 20 days of inoculation) on different level of BA in *C.setigerus* has been presented in table – 4.16.

Callus induction frequency revealed nonsignificant difference among varying level of BA in seed explant of *C.setigerus*. The average callus induction frequency was 49.36%. Maximum callus induction frequency (59.02%) was noticed on without (0.00 mg/l) BA (Plate # 3, fig. 2) which was at par with media containing 0.5 mg/l BA (58.52%) and 0.5 mg/l BA+500 mg/l CH (53.54%). The effect of BA at the level of 0.2 mg/l concentration on callus induction was observed to be the poorest (26.38%).

In case of callus induction from immature inflorescence explant of *C.setigerus* also, there was no significant difference among the media with varying concentration of BA. The average response for callus induction from inflorescence explant was 35.79%.

The highest induction in callus was shown by the medium containing 0.5 mg.l BA (64.28%) and the least callus induction performance (22.22%) was

recorded on the media containing 0.2 mg/l BA followed by the culturing media without BA (23.23%). The culture medium containing 0.5 mg/l BA+500 mg/l CH exhibited moderate response for callus induction (33.33%).

(c) *C.echinatus:*

The effect of BA in terms of response for callus induction from the seed explant of *C.echinatus* is shown in table 4.17.

No significant difference was found among different BA levels applied to the culture medium. The average callus induction response as figured in the table was 42.53%. Culture media with 0.5 mg/l BA+500 mg/l CH exhibited highest callus induction response (49.62%) among all the combinations of BA concentrations applied. This was followed by the medium without BA (64.2%) and the culture medium supplemented with 0.2 mg/l (44.44%). The culture medium containing 0.5 mg/l BA was least responsive in terms of callus induction (30.87%) among all the BA concentrations.

Same treatments were applied for the immature inflorescence explant but no callus induction response from this explant occurred on the media either with different concentrations of 2,4-D or with different concentrations of BA or kin.

(B) Quality

(a) *C.ciliaris:*

(i) Seed explant

The effect of different concentration of BA in MS basal medium suplplemented with 3.0 mg/l 2,4-D on colour and texture of callus induced from the seed explants of six genotypes of *C.ciliaris* is presented in table - 4.18

With regard to callus colour and texture, there was significant difference among different combinations of media. No significant difference was found among the media containing 0.50 mg/l BA and media containing 0.5 mg/l BA+500 mg/l CH, which showed best score for callus quality. Both of these media significantly differed from the medium devoid of BA. Media with 0.2 mg/l BA showed intermediate response in terms of callus quality.

With respect to the genotypes, no significant differece was observed among them for callus colour and texture. The maximum values were exhibited by the genotypes EC397680 for both of the characters (yellowish green colour, 1.88

Table 4.18: Effect of different concentrations of BA on colour and texture of callus induced from seed explant of six genotypes of *C.ciliaris*

Genotype	BA concentrations (mg/l) with 3.0 mg/l 2,4-D									
C.ciliaris	0.00		0.20		0.50		0.50+500 mg/l CH		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	0.75	0.45	1.87	1.25	1.50	2.12	2.00	2.07	1.53	1.47
EC400587	1.00	0.72	2.00	1.11	2.25	1.83	1.87	2.52	1.78	1.54
EC397600	0.63	0.95	1.50	1.77	1.87	2.17	1.62	2.00	1.40	1.72
EC400610	0.44	0.67	1.25	1.75	1.70	2.10	1.14	2.02	1.13	1.63
EC397680	0.90	1.62	1.37	2.35	2.50	2.87	2.75	2.82	1.88	2.41
IG693108	0.83	1.27	1.30	1.70	2.09	2.30	2.47	2.11	1.67	1.84
Mean	0.75	0.94	1.54	1.65	1.98	2.23	1.97	2.26		

	Genotype		Media	
	Colour	Texture	Colour	Texture
KW	5.65	9.41	26.29	26.78
Probability	0.34	0.09	0.00	0.00

Table 4.19: Effect of different concentrations of BA on colour and texture of callus induced from immature inflorescence explants of six genotypes of *C.ciliaris*

Genotype	BA concentrations (mg/l) with 3.0 mg/l 2,4-D									
C.ciliaris	0.00		0.20		0.50		0.50+500 mg/l CH		Mean	
	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture	Colour	Texture
EC400631	0.87	0.67	1.92	2.70	2.62	2.80	2.75	2.55	2.04	2.18
EC400587	0.90	0.86	1.95	1.97	2.25	2.12	1.87	2.30	1.74	1.81
EC397600	1.12	1.05	1.85	2.47	2.00	2.43	1.70	2.40	1.66	2.08
EC400610	0.80	0.72	1.83	2.00	1.99	2.25	2.12	2.37	1.68	1.83
EC397680	0.80	0.57	1.25	1.12	1.95	1.37	2.00	1.37	1.50	1.10
IG693108	0.67	0.62	1.44	1.74	1.27	2.02	1.37	2.10	1.18	1.62
Mean	0.86	0.74	1.70	2.00	2.01	2.16	1.96	2.18		

	Genotype		Media	
	Colour	Texture	Colour	Texture
KW	8.75	7.49	22.53	21.55
Probability	0.11	0.18	0.00	0.00

Table 4.20: Effect of varying levels of BA on colour and texture of callus induced from different explants of *C.setigerus*

Concentrations of BA (mg/l) with 3.0 mg/l 2,4-D	Explant			
	Seed		Inflorescence	
	Colour	Texture	Colour	Texture
0.00	1.50	0.77	0.87	0.80
0.20	2.00	1.78	2.00	1.92
0.50	2.30	1.75	1.87	1.75
0.50+500 mg/l CH	1.87	2.12	2.12	1.84
KW	3.33	4.50	4.34	4.09
Probability	0.34	0.21	0.22	0.25

Table 4.21: Average performance of *C.ciliaris*, *C.setigerus* and *C.echinatus* for callus induction frequency on different media

	Seed explant		Immature inflorescence explant	
	2,4-D	BA	2,4-D	BA
C.ciliaris	53.8**	54.43**	28.89**	41.46
C.setigerus	20.69	49.36	17.17	35.79
C.echinatus	48.49	42.53	0	0

and granular type of callus, 2.41). For callus colour also EC400587 performed fairly good (1.78) followed by the IG693108 (1.67) and EC 400631 (1.53). The genotypes EC400610 and EC397600 showed comparatively poor response with 1.13 and 1.40 colour scores, respectively. For callus texture the genotype EC397680 performed best with the score 2.41 followed by the genotype IG693108 (1.84), EC397600 (1.72) and EC400610 (1.63). Comparatively poor texture was observed in the genotypes EC400587 (1.54) and EC400631 (1.47).

(ii) **Immature inflorescence explant**

The effect of different levels of BA on colour and texture of callus induced from immature inflorescence explants of *C.ciliaris* is presented in table - 4.19.

The effect of media was significantly different for callus texture and colour. Media supplemented with 0.5 mg/l BA and 0.5 mg/l BA+500 mg/l CH did not show significant difference between them. Both were almost similar for callus colour and texture and exhibited best performance followed by the media containing 0.2 mg/l BA. The media devoid of BA was found to be the poorest among all and showed significant difference from the media containing 0.5 mg/l BA+500 mg/l CH.

Genotype showed non-significant difference among them. The highest performance for average value of callus colour was shown in the genotype EC400631 (2.04, greenish) which was followed by the genotype EC400587 (1.74). EC400610 (1.68) and EC397600 (1.66) were similar in performance with yellowish green colour of callus. Minimum score for callus colour was recorded for the genotype IG693108 (1.18) followed by EC397680 (1.50) with yellow colour of callus. With regard to callus texture, the best performing genotype was EC400631 (2.18) with granular type of callus which was comparable to the genotype EC397600 (2.08). EC400610 (1.83) and EC40587 (1.81) were comparable to each other with friable to granular texture of callus. The least scoring type of callus texture was observed in the genotype EC397680 (1.10). The genotype IG693108 was found to be better (1.62) than this genotype for callus texture.

(b) *C.setigerus*

MS basal medium supplemented with 3.0 mg/l 2,4-D was used and the effect of different concentration of BA for colour and texture of callus induced from seed and immature inflorescence explants of *C.setigerus* was observed. The data are presented in table – 4.20.

Non-significant difference was observed for colour as well as texture in both the explants. However, the medium supplemented with 0.5 mg/l BA for colour (2.30) and the media containing 0.5 mg/l BA+500 mg/l CH for texture (2.12) in callus from seed explant and media containing 0.5 mg/l BA+500 mg/l CH for colour (2.1) and media containing 0.2 mg/l BA for texture of callus induced from immature inflorescence explant were found to be the best. Absenceof BA showed poorest effect on the callus quality both for colour and texture in *C.setigerus*.

(c) *C.echinatus*

The effect of different concentrations of BA on colour and texture of calli are described in clause 4.1.1.1Bc and table - 4.06

4.1.1.4 Comparative performance of *Cenchrus* species for callus induction:

The average performance of callus induction frequency from seed and immature inflorescence explants among *C.ciliaris, C.setigerus* and *C.echinatus* as influenced by varying concentrations of 2,4-D has been presented in table 4.21.

The effect of 2,4-D in *C.ciliaris* was highly significant for both the explants, while the effect of BA was highly significant for callus induction response from the seed explant only. The mean values of callus induction frequency revealed that there was higher callus induction response from seed explant as influenced by different BA levels than that with the different 2,4-D levels in the same explant. Callus induction response from inflorescence explant also was at a lesser frequency with varying level of 2,4-D than with different levels of BA, similar to the response with seed explant.

In case of *C.setigerus*, none of the hormone level was significant for the explants. In this species also the varying levels of BA exhibited callus induction frequency better than those with varying concentrations of 2,4-D in both the explants.

In *C.echinatus*, the callus induction response exhibited by the seed explant only and the effects of 2,4-D and BA were not significant. In case of *C.echinatus*, varying levels of 2,4-D were found better than the varying levels of BA in terms of callus induction frequency which was contrary to the response exhibited by *C.ciliaris* and *C.setigerus*.

On comparing the callus induction frequency from the seed and inflorescence explants among the three species, *C.ciliaris* excelled for both the explant followed by *C.setigerus*. However, for the average callus induction frequency from the seed explant as influenced by varying concentrations of 2,4-D, *C.echinatus* excelled over that of *C.setigerus*.

4.1.2 Callus Growth

(i) Seed Explant

The data in growth rate in terms of per cent growth of callus weight observed at the interval of 20-21 days up to the third passage of the subculture of calli from seed explant of all the eight genotypes of the three *Cenchrus* species on the three different basal media MS, SH and B_5 supplemented with 0.2 mg/l BA and 3.0 mg/l 2,4-D used for all the three passages of subcultures are present in fig - 1a.

Among all the genotype, EC400587 of *C.ciliaris* exhibited the highest growth rate in all the MS (Plate # 4, fig.1), SH and B_5 media. However, *C.setigerus* performed best among all the genotypes for callus growth on B_5 media and it was at par with the performance of EC400587 of *C.ciliaris* on MS and SH media in the first passage. Lowest callus growth was exhibited by *C.echinatus* (EC397342) on all the three media.

Among all the different culture media used, MS medium in general was found to be the best for higher callus multiplication for all the genotypes of *C.ciliaris* (Plate # 4, Fig. 1 and Fig. 2) as compared to SH asnd B_5 medium. For *C.setigerus* (EC400639) and *C.echinatus* (EC397342) (Plate # 4, Fig. 3), highest callus growth was noticed with B_5 media in first passage only, but in

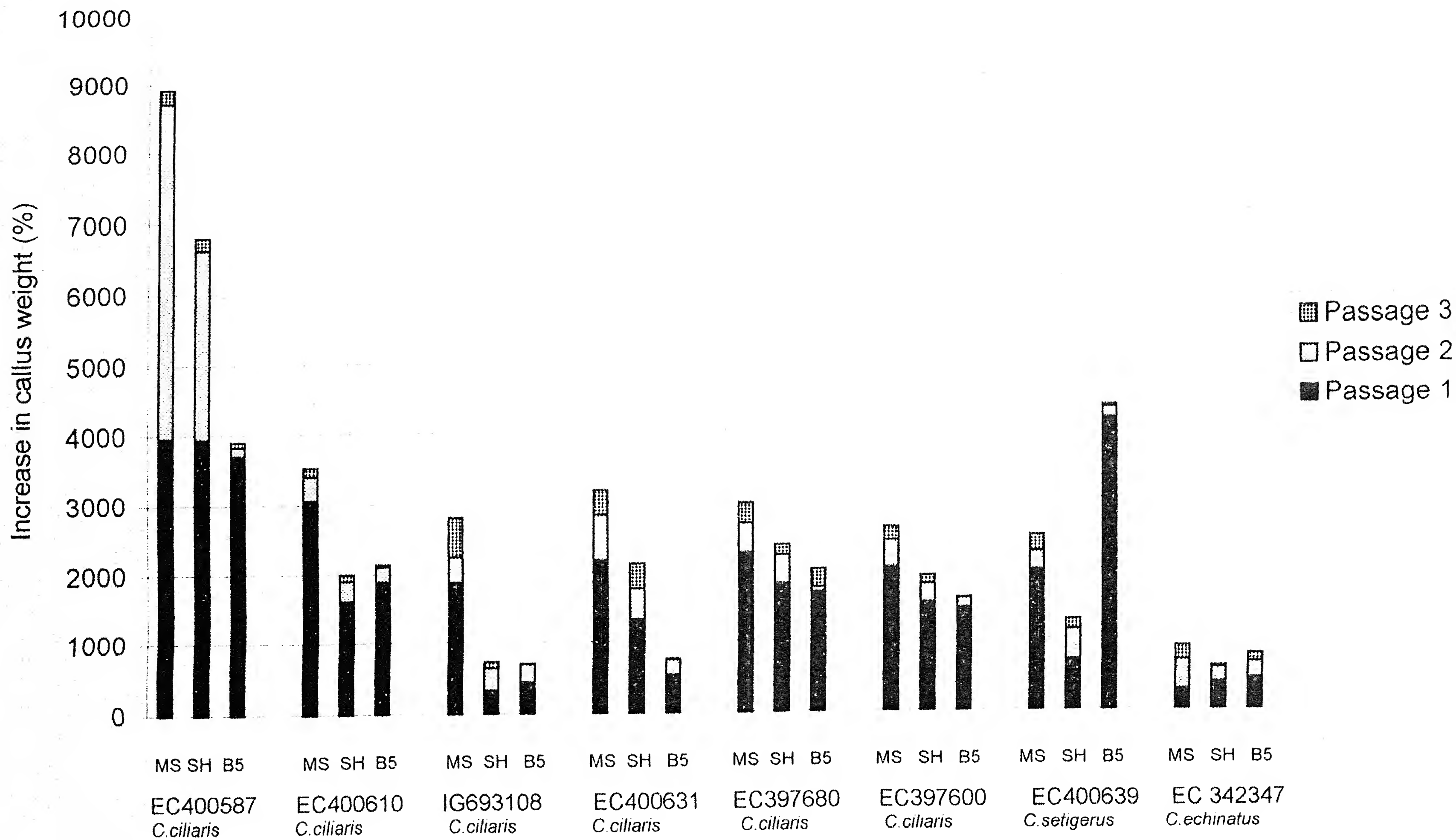


Fig 1a: Callus growth rate from seed explant in eight *Cenchrus* genotypes

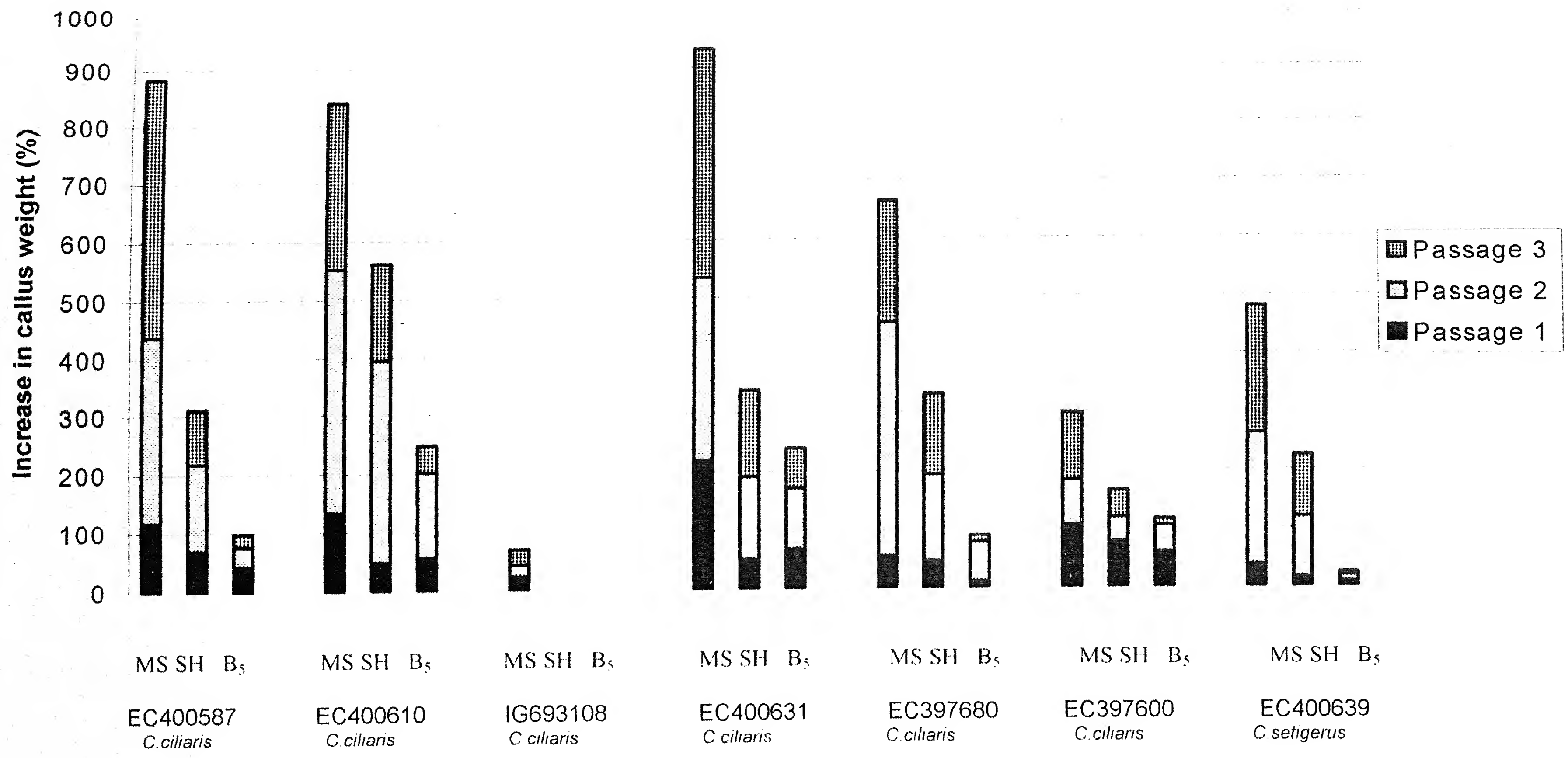


Fig 1b: Callus growth rate from immature inflorescence explant in eight *Cenchrus* genotypes

rest of the passages MS and SH media performed better than B5. The performance of MS medium for callus growth rate was much pronounced during second passage also in all the genotypes except in case of *C.setigerus* (EC400639) where growth rate in second passage was comparatively better on SH medium. *C.ciliaris* (EC400587) and *C.echinatus* (EC397342) showed higher growth rate of callus in second passage as compared with that in first passage on MS medium. Maximum callus growth rate in the second passage of subculture was observed on MS medium in EC400587 followed by that on SH medium in the same genotype of *C.ciliaris*. In general growth rate decreased with the advancement of subculturing. All the genotypes showed comparatively less growth rate with subsequent passage except EC400587 of *C.ciliaris* and *C.ehinatus* (EC397342) on MS medium. In the third passage callus growth was drastically decrease in all the genotypes except in IG693108 of *C.ciliaris*. In this genotype, on MS medium, the callus growth rate was higher than in the third passage of subculture than the second passage. In the genotype EC400631 of *C.ciliaris* also, the growth rate of calli was quite satisfactory in the third passage of subculture both on MS and SH media which was at par. For the third passage in all the genotypes, MS medium in general performed best for callus growth followed by SH medium. The growth rate during the third passage was recorded poorest on B_5 medium among all the genotypes.

(ii) Immature inflorescence explant

The comparative data on growth rate in terms of per cent growth of callus weight as observed at 20-21 days of interval upto the third passage of subculture of calli from immature inflorescence explant of all the eight genotypes of the three *Cenchrus* species on three different culture media are presented in fig-1b. The basal media, MS, SH and B_5 used for all the three passages of subcultures were supplemented with 3.0 mg/l 2,4-D and 0.2 mg/l BA. Among all the genotypes, EC400631 of *C.ciliaris* showed maximum callus multiplication on MS medium, which was at par with EC400587 and EC400610 of *C.ciliaris* on the same medium. Minimum callus production response on MS medium was recorded in IG693108 of *C.ciliaris*. This

genotype of *C.ciliaris* responded only on MS medium and its performance for callus production was poorest in all the passages. It did not exhibit any callus production on SH or B_5 medium. Rest of the genotypes of *C.ciliaris* (EC397680 and EC397600) and *C.setigerus* (EC400639) were in medium range in terms of callus multiplication.

MS medium was found to be the best among all the three media since all the genotyupes on MS medium showed maximum callus multiplication followed by the SH medium while minimum callus multiplication was recorded on B_5 medium. SH medium showed best callus growth in EC400610 followed by EC397680 and EC400631 which were at par. The minimum callus growth on the same medium was recorded in EC397600. Similar to SH, B_5 medium also showed maximum callus growth in EC400610 followed by EC400631 both of *C.ciliaris* which were at par, where as the maximum callus growth on B_5 medium was recorded in EC400639 of *C.setigerus* (Plate # 5 Fig. 2). However, there was no response at all for callus induction and growth as observed from inflorescence explant of *C.echinatus* (EC397342) on any of the three media, hence not represented in fig. 1b.

With respect to the passages, callus growth rate in first passage was comparatively less than the second and third passages in general among all the genotypes which were contrary to the callus growth pattern from seed explant as shown in fig-1a. However, in case of the genotype EC397600 on SH and B_5 and in case of EC400587 on B_5 medium, the comparative callus growth rate in the first passage was observed more than second and third passages.

The maximum callus growth in the second passage of subculture was recorded in EC400610 on MS medium followed by the same genotype on SH medium and EC400587 and EC400631 on MS medium, which were at par. The maximum growth was on B_5 medium in second passage was observed in EC400610 followed by EC400631 of *C.ciliaris*. Thus EC400610 exhibited maximum callus production rate in all the three media during second passage of subculture. The minimum growth rate on MS, SH and B5 media was recorded in IG693108, EC397600 both of *C.ciliaris* and EC400639 of *C.setigerus*, respectively.

Plate # 2

Seed explant

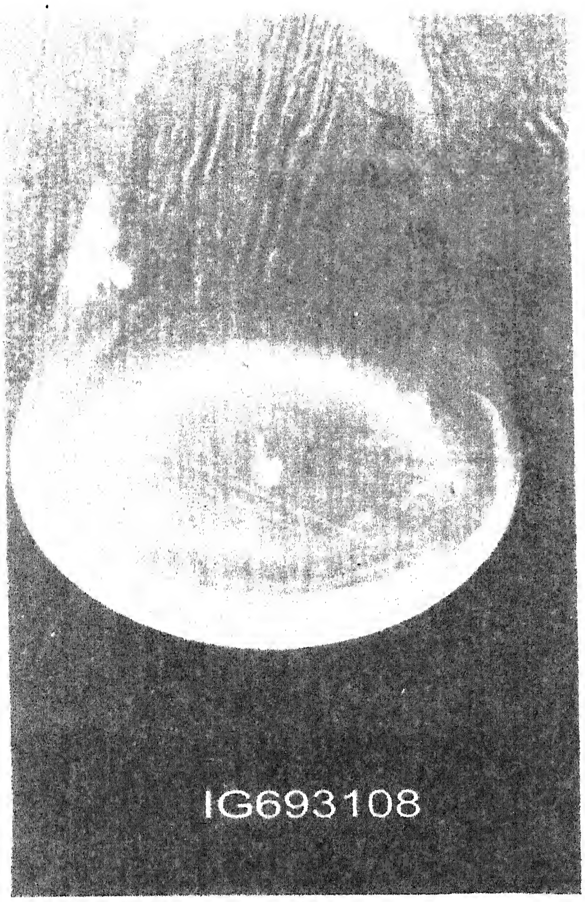


fig-1 Callus induction
(C. ciliaris)

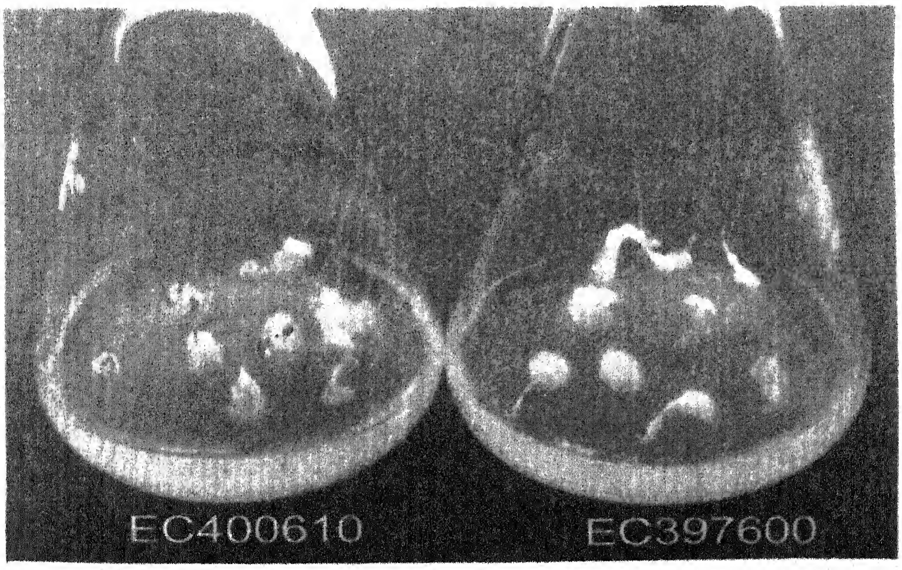


fig-2 Callus induction and development *(C. ciliaris)*

Plate # 3

Callus Induction from seed explant

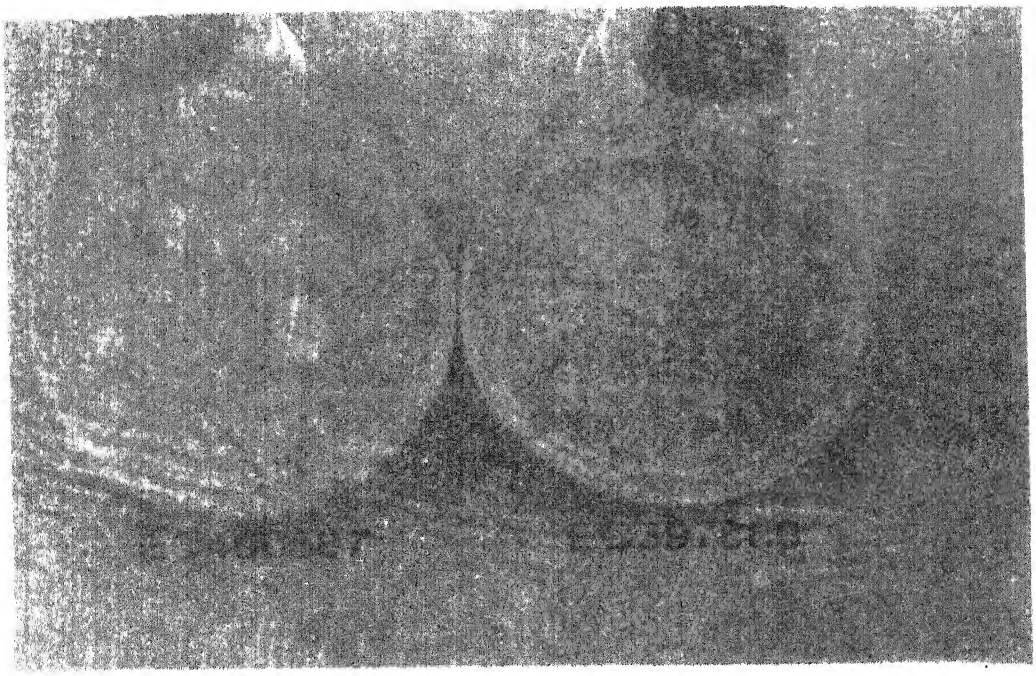


fig-1 Callus induction in *(C. ciliaris)*

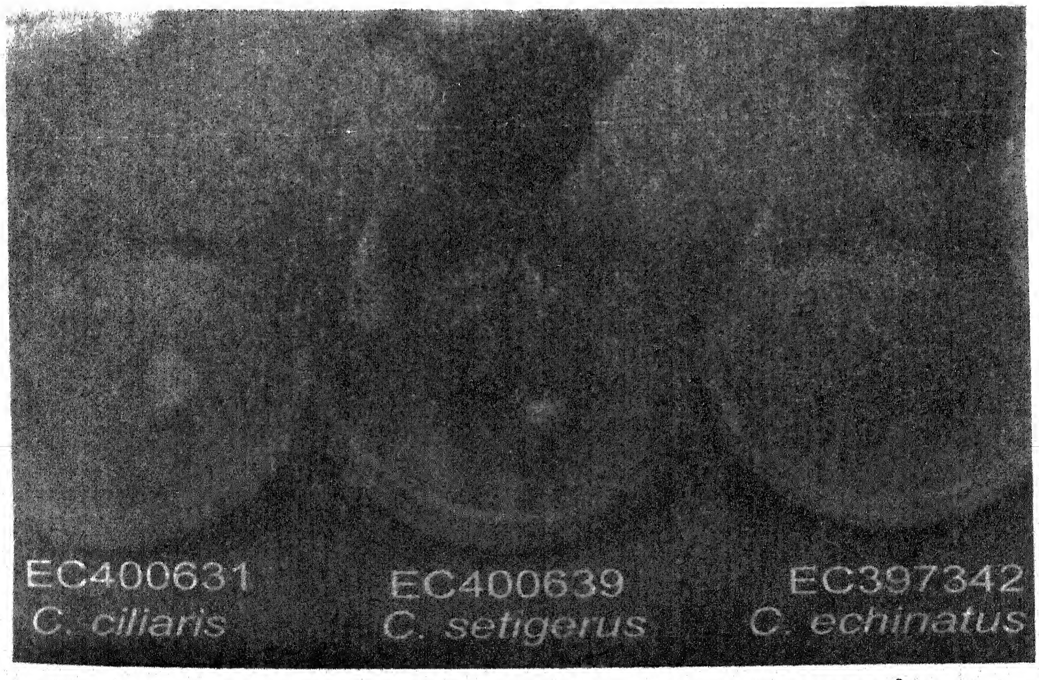


fig -2 Callus induction in *Cenchrus* species

Plate # 4

Callus Growth

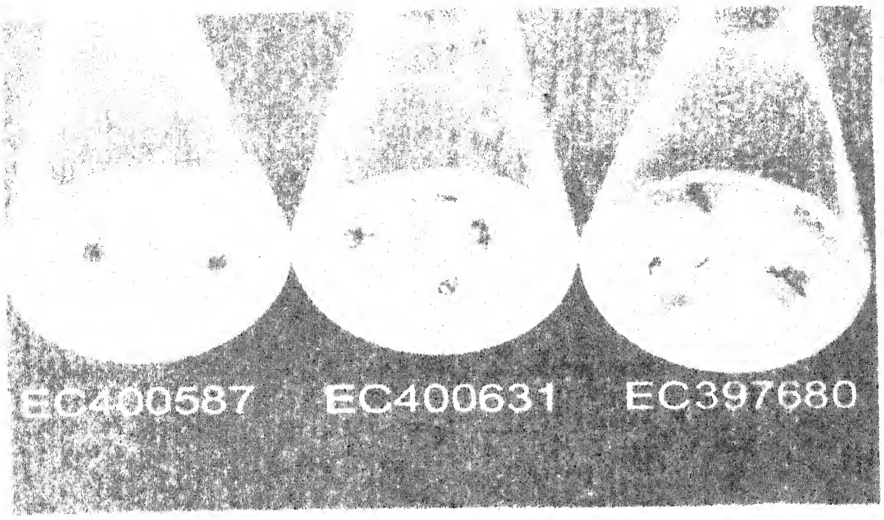


fig-1 *C.ciliaris*

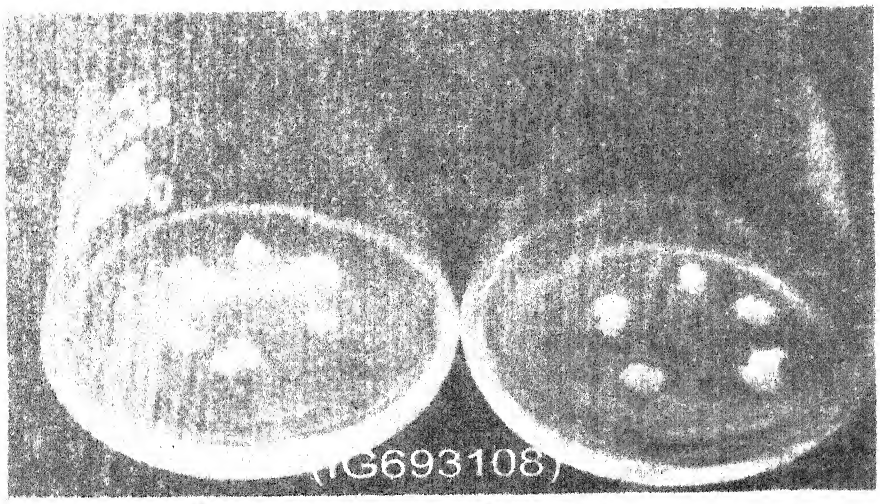


fig-2 *C.ciliaris*

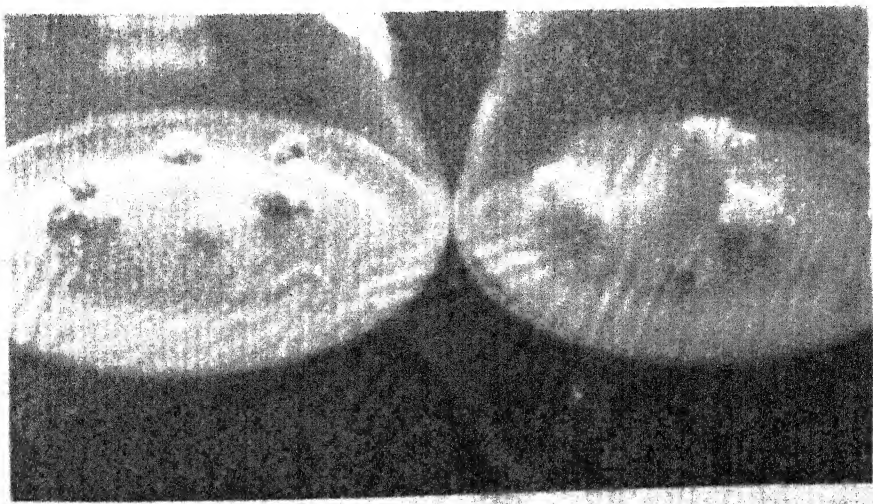


fig-3 *C. setigerus* *C.echinatus*

Plate # 5

Inflorescence explant

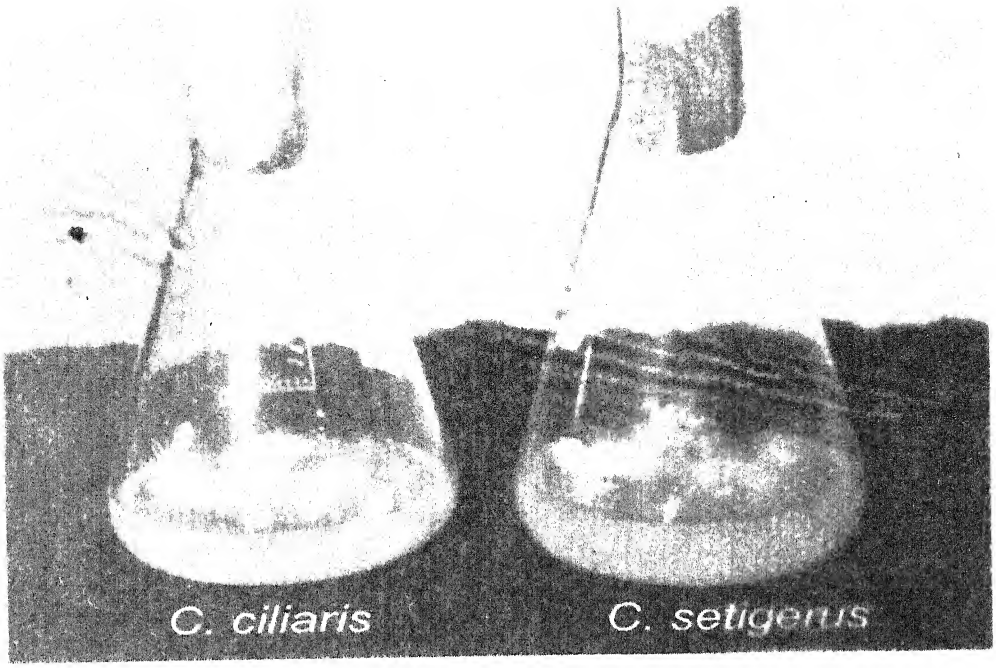


fig-1 Callus induction

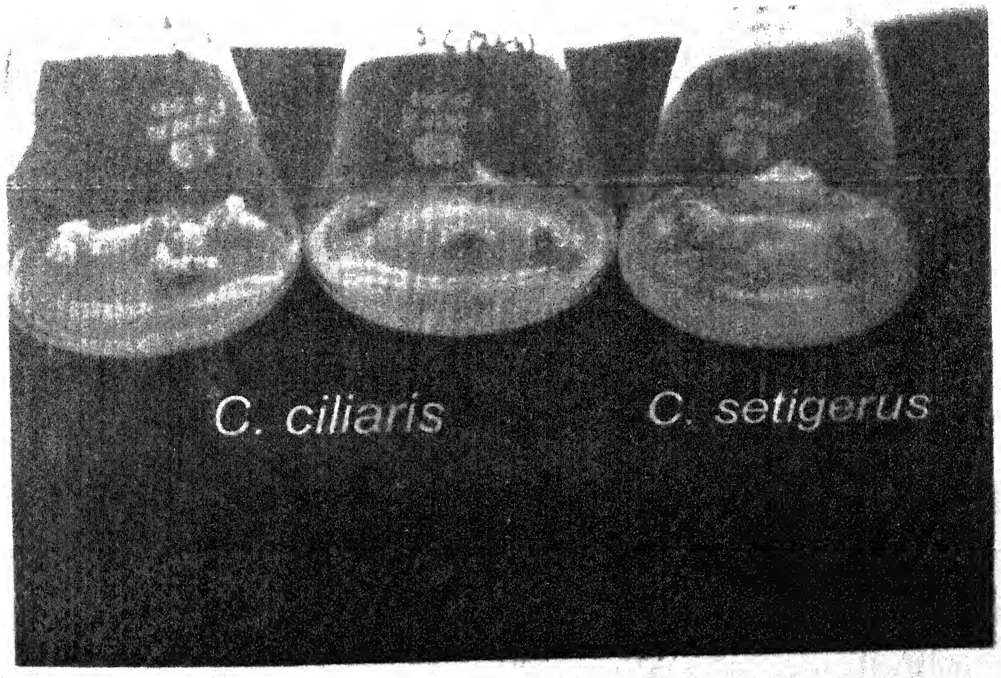


fig-2 Callus Development

Plate # 6

Colour and Texture
of
Callus

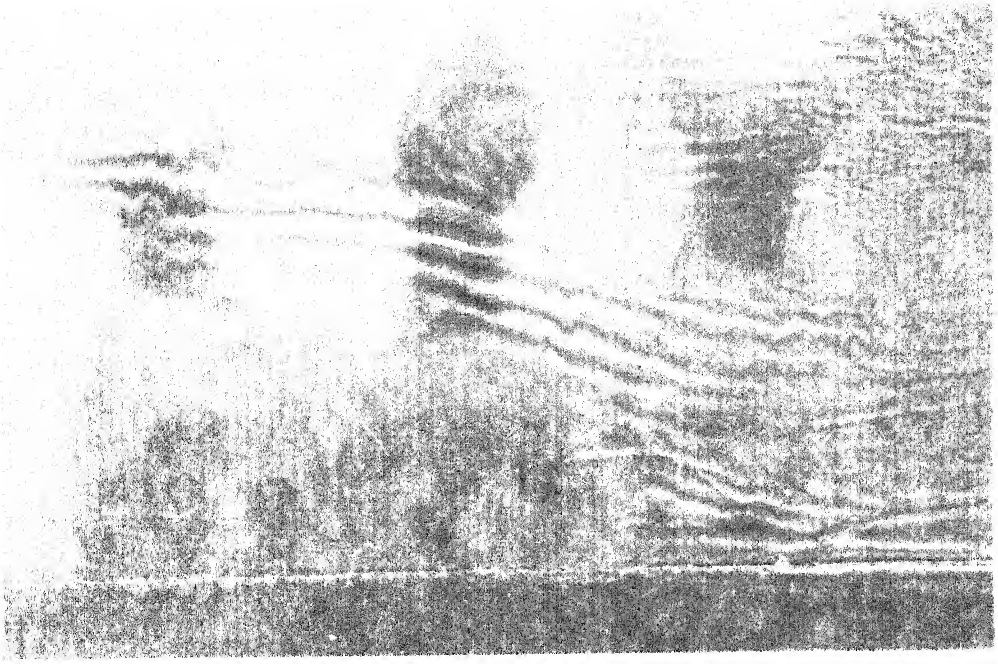


fig-1 Brown and Vitrious callus

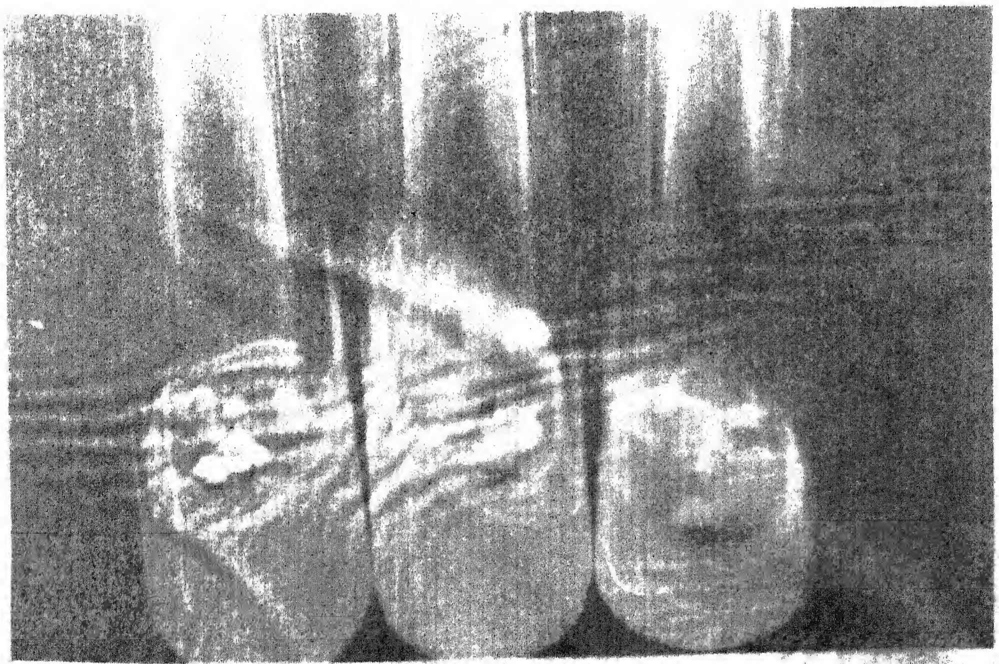


fig-2 Yellowish, fragile callus

Plate # 7

Colour and Texture
of
Callus

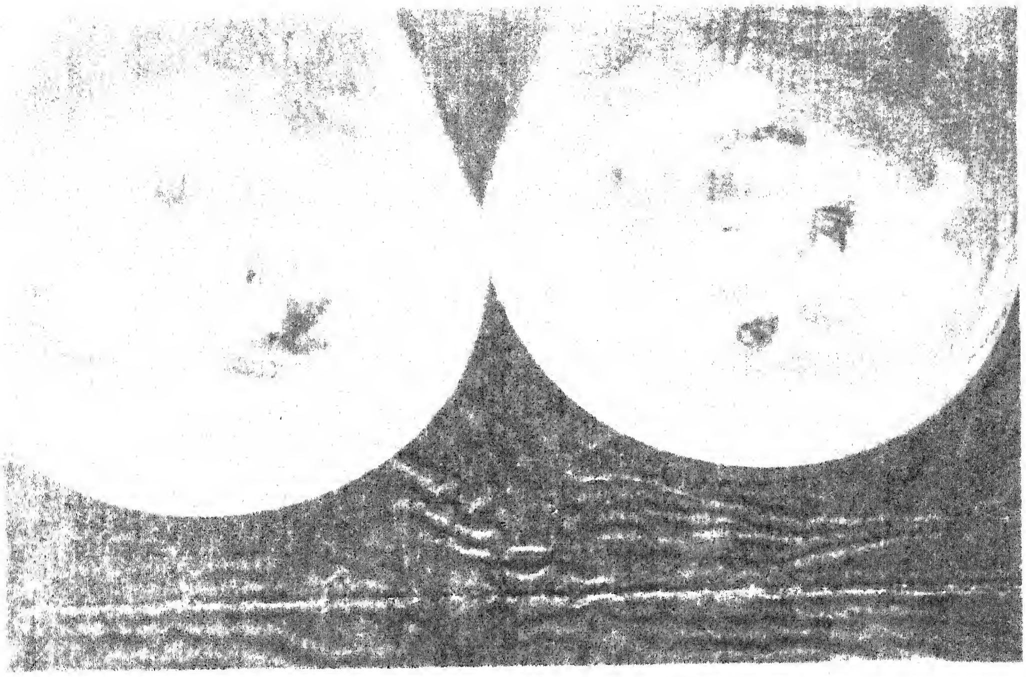


fig-1 Friable, white callus

fig-2 Greenish - white, friable
and Compact callus

In the thirs passage of subculture, the callus growth rate was found maximum on MS medium in all the genotypes followed by on SH medium and it was found minimum on B_5 medium. EC400587 exhibited maximum callus growth rate followed by EC400631 and the minimum growth rate was recorded in IG693108. On SH medium the maximum callus growth in the third passage was observed in EC400610 followed by EC400631 and EC397680 (Plate # 4, fig. 1) which were at par. The minimum rate of growth was exhibited by EC397600. The best callus growth on B_5 medium in the third passage of subculture was observed in EC400631 followed by EC400610, both of *C.ciliaris* and the minimum growth rate was represented by EC400639 of *C.setigerus*.

The growth rate of callus in the third passage over to that in the second passage of subculture was observed to be higher in the genotype EC400587, EC400631, EC397680 and EC397600, all of *C.ciliaris*, on MS medium. In all these genotypes on the other two media and in the rest of the other genotypes on all the three media, the growth rate showed a declining trend from second to the third passage of subculture except in case of EC400639 of *C.setigerus* where the callus growth rate remained at par from second to third passage of subculture in all the three culture media (Plate # 5, Fig. 2).

4.1.3 Regeneration

It has been mentioned in the results of previous experiments that intitiation of callus was observed from the seed and immature inflorescence explants of different genotypes of *Cenchrus* species except the immature inflorescence explant of *C.echinatus*. The nature of calli, their growrh rate, texture and colour differed and were dependent on organs or parts of the genotypes from which they were derived. These calli were maintained on MS medium supplemented wity 3.0 mg/l 2,4-D and 0.2mg/l BA or kinetin.

4.1.3.1 Morphogenetic response of calli

The experiments were designed to induce morphogenetic response for differentiation in these callus cultures. The calli raised from different organs/parts of various genotypes were transferred from callusing media to different regeneration media, with several combonations of growth hormones. The regenrerability of different types of calli was tested and it was observed that only compact white and nodular calli were capable of regeneration. After 8-10 days of transfer to the regeneration media, these calli started differentiation towards regeneration. The most frequently regeneration response noted was the organogenic induction of shoot buds. Multiple shoot bud formation also occurred very frequently. Intermittent occurrence of somatic embryo development was also observed which was rare and quite sporadic.

(A) Histology

The nature of shoot bud organogenesis, multiple shoot bud initiation and somatic embryogenesis were also examined histologically under the compound microscope after fixing, microtome sectioning and staining with safranin and fast-green of the regenerating calli in which the differentiation was initiated. It was observed that the potentially regenerating calli were composed of compact cell masses of comparatively uniform and smaller size with densely staining cytoplasm and prominent nucleus intermingled with high starch granule containing cells and these regenerating cells were without intercellular spaces and exhibited meristematic activity (plate # 11, fig. 1, 2). On the contrary the vitreous and othere nonregenerating types of calli were invariably composed of loose masses of cells of various dimensions and shapes ranging from vesicular to syphonous/polysyphonous type. These cells were very weakly staining with feeble cytoplasm and containing obscure nucleus or were even without any nyucleus (plate # 11, fig. 3).

The differentiating shoot buds during organogenesis exhibited typical apical shoot bud structures having clearly discernible tunica-corpus organization and with subtending leaf promprdia (plate # 12, fig. 1). In multiple shoot structures the apical dome was akin to that of transition dome like structures where each dome represented individual vegetative shoot bud dome bearing leaf primordia laterally

(plate # 12, fig.2). These individual apical domes also exhibited typical tunica-corpus type of organization supporting for their vegetative shoot bud nature. The somatic embryos showed bipolar organization and exhibited the structure of a typical monocot type of embryo representing coleptile region towards the shoot primordial end and coleorhizal region towards the root primordial end of the somatic embryos. The scutellar region was observed in the lateral region of the somatic embryo. (plate # 12, fig. 3)

After the onset of regeneration the cultures were transferred from dark to diffused light conditions as the calli subjected to the exposure of light prior to onset of regeneration led to browning of the calli followed by their death. Greening of the regenerating callus mass and initiation of leaf development on the organogenic calli was observed within two weeks of their transfer to regenerating media. At this stage, the cultures were transferred to 16 hours lighty/8 hours dark photoperiod at 2500 lux light intensity for further development of regenerating shoots.

(B) Shoot bud organogenesis

(i) *C. ciliaris*

Shoot bud organogenesis in terms of regeneration frequency of shoot buds was observed in differentiating calli derived from seed explants of various genotypes of *C. ciliaris*. Effect of different levels of cytokinins and the effect of application of 2.4-D on six genotypes of *C.ciliaris* showed highly significant difference among them with regard to regeneration frequency (expressed as number shoots/100 mg calli) from seed explant derived callus cultures (table 4.22). Best performance was observed in the genotype EC400610 (0.86 shoots/100 mg calli) (plate # 8, fig 2) which was at par with IG693108 (0.73) (plate # 8,fig. 3) followed by EC397680 (plate # 9, fig. 1) (0.60) and EC400587 (0.55) (plate # 8, fig. 1). Minimum response for the regeneration was recorded in EC400631 (0.17). However, EC367600 (plate # 9, fig. 1) was far better than EC400631 in terms of regeneration frequency with 0.44 shoots/100 mg of callus.

With respect to media, highly significant difference was found for regeneration potential. The data revealed that the level of kin and BA both applied at the rate of 2 mg/1 was found better than the other concentrations of both the cytokinins

Table 4.22: Effect of different cytokinins on regeneration frequency from seed callus cultures of the six genotypes of *C.ciliaris*

Genotypes	MEDIA								
	With 0.2 mg/l 2,4-D						Without 2,4-D		
	Kinetin (mg/l)			BA (mg/l)			Kinetin	BA	Mean
	2.0	3.0	5.0	2.0	3.0	5.0	2.0	2.0	
EC 397600	1.63	0.21	0.53	0.38	0.00	0.40	0.11	0.78	0.44 bc
EC397680	0.81	0.66	0.32	1.72	0.34	0.26	0.48	0.22	0.60 abc
EC 400631	0.24	0.11	0.12	0.49	0.56	0.10	0.63	0.18	0.17 c
EC400587	0.45	0.85	0.14	0.74	1.16	0.22	0.36	0.57	0.55 abc
EC400610	1.78	0.83	0.26	2.0	0.35	0.65	0.76	0.27	0.86 a
IG693108	1.44	0.37	0.14	1.24	1.42	0.15	0.79	0.32	0.73 ab
Mean	1.06 a	0.50 ab	0.17 c	1.09 a	0.55 ab	0.30 bc	0.43 abc	0.38 bc	

F test	
Genotype	3.57**
Media	5.34**
Interaction	1.16

Table 4.23: Response of different cytokinins on regeneration frequency from immature inflorescence derived callus cultures in the six genotypes of *C.ciliaris*

Genotypes	MEDIA								
	With 0.2 mg/l 2,4-D						Without 2,4-D		
	Kinetin (mg/l)			BA (mg/l)			Kinetin	BA	Mean
	2.0	3.0	5.0	2.0	3.0	5.0	2.0	2.0	
EC 397600	025	0.43	0.39	0.16	0.53	0.36	0.20	0.29	0.32 c
EC397680	0.38	0.27	0.36	0.54	0.16	0.19	0.14	0.92	0.37 bc
EC 400631	2.72	0.48	0.47	2.10	0.47	0.66	0.47	0.25	0.95 a
EC400587	0.86	0.85	0.43	1.82	0.22	0.46	0.48	0.43	0.69 ab
EC400610	1.85	0.46	0.50	1.32	0.33	0.55	1.04	0.71	0.84 a
IG693108	1.27	0.59	0.40	0.99	0.36	0.18	0.78	0.26	0.60 b
Mean	1.22 a	0.51 b	0.42 abc	1.23 a	0.26 c	0.40 bc	0.52 b	0.48 b	

F test
Genotype 9.05**
Media 15.15**
Interaction 2.74**

Table 4.24: The effect of cytokinins on regeneration frequency (No. of shoots /100 mg calli) from callus cultures of *C.setigerus*

Media	Explant	
MS with different concentrations of cytokinin (mg/l)	Seed	Inflorescence
with 0.2 mg/l 2,4-D		
2.0 kinetin	0.90	0.70
3.0 kinetin	0.11	0.42
5.0 kinetin	0.13	0.32
2.0 BA	0.72	0.83
3.0 BA	0.17	0.33
5.0 BA	0.04	0.21
Without 2,4-D		
2.0 kinetin	0.85	0.21
2.0 BA	0.10	0.13
Mean	0.37	0.39
F value	1.60	1.25

Table 4.25: The effect of cytokinins on regeneration frequency (No.of shoots/100 mg calli) from callus cultures of *C.echinatus*

MS basal medias with different concentrations of cytokinin (mg/l)	Seed explant
With 0.2 mg/l 2,4-D	
2.0 kinetin	0.16
3.0 kinetin	0.24
5.0 kinetin	0.09
2.0 BA	0.12
3.0 BA	0.16
5.0 BA	0.20
Without 2,4-D	
2.0 kinetin	0.84
2.0 BA	0.18
Mean	0.24
F value	1.72

along with 0.2 mg/l 2,4-D. The media contained 2.0 mg/l BA +0.2 mg/l 2,4-D exhibited best regeneration response (1.09) which was at par with the media containing 2 mg/l kin + 0.2 mg/l 2,4-D (1.06). The media having more cytokinins alone than 2.0 mg/l did not perform so good for regeneration. The other regeneration media did not perform as good as these two media contained 2,4-D @ 0.2 mg/l for regeneration. Remaining media combinations exhibited lower regeneration frequency. Regeneration frequency on the medium containing 3.0 mg/l BA+0.2 mg/l 2,4 –D (0.55) was at par with 3.0 mg/l kin+0.2 mg/l 2,4-D (0.50) followed by the media containing 2.0 mg/l kin alone (0.43) and 5.0 mg/l BA+0.2 mg/l 2,4-D (0.30). The poorest performance was recorded on media containing 5.0 mg/l kin+0.2 mg/l 2,4-D for regeneration.

Interaction between media and genotypes was not significant. The highest regeneration was observed in EC400610 (2.0) on media having 2.0 mg/l BA+0.2 mg/l 2,4-D and the lowest was shown by the genotype EC397600 (0.00) on the media containing 3.0 mg/l BA+0.2 mg/l 2,4-D. Supplementation of auxin (2,4-D) at the rate of 0.2 mg/l markedly increased the regeneration frequency as shown by the data taken at 2.0 mg/l of cytokinins.

The regeneration frequency (# shoot/100 mg calli) in six genotypes of *C.ciliaris* from the callus cultures of inflorescence is presented in table-4.23. The genotypes showed highly significant difference among them in terms of regeneration potential. Best performing genotype was EC400631 (plate # 8, fig. 2) with 0.95 shoot/100 mg of callus which was at par with EC400610 (0.84) and followed by the genotype EC400587 and IG693108 with 0.69 and 0.60 shoots/100 mg of callus, respectively. The least regeneration frequency was observed in the genotype EC397680 (0.37).

There was highly significant difference among all the media tried for regeneration. The data revealed that the application of kinetin/BA at the level of 2.0 mg/l gave best response in terms of regeneration potential from calli derived from inflorescence explant of *C.ciliaris*. Media supplemented with 0.2 mg/l 2,4-D enhanced the regeneration capacity when applied with 2.0 mg/l kin or BA as shown in the table. Regeneration frequency was almost similar on 2.0

Plate # 8

Regeneration in *C.ciliaris*

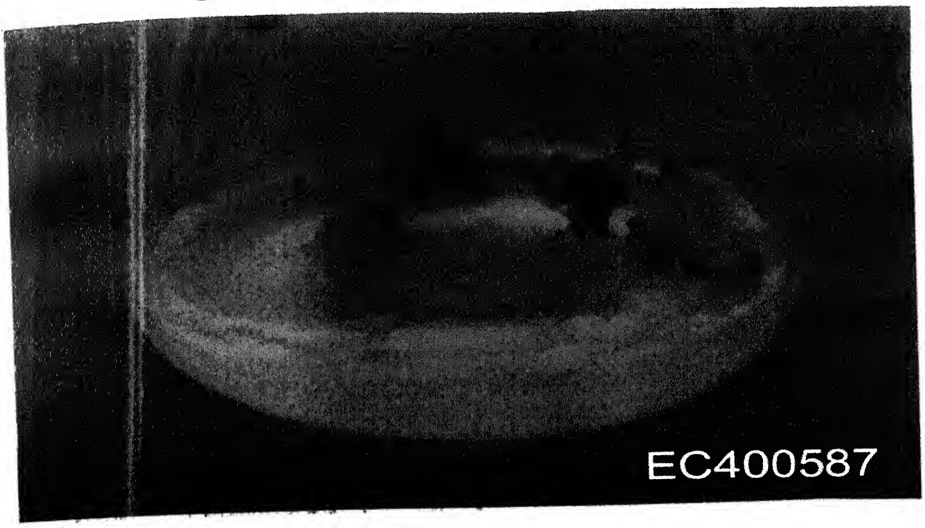


fig-1 Shoot induction

fig-2 Shoot induction

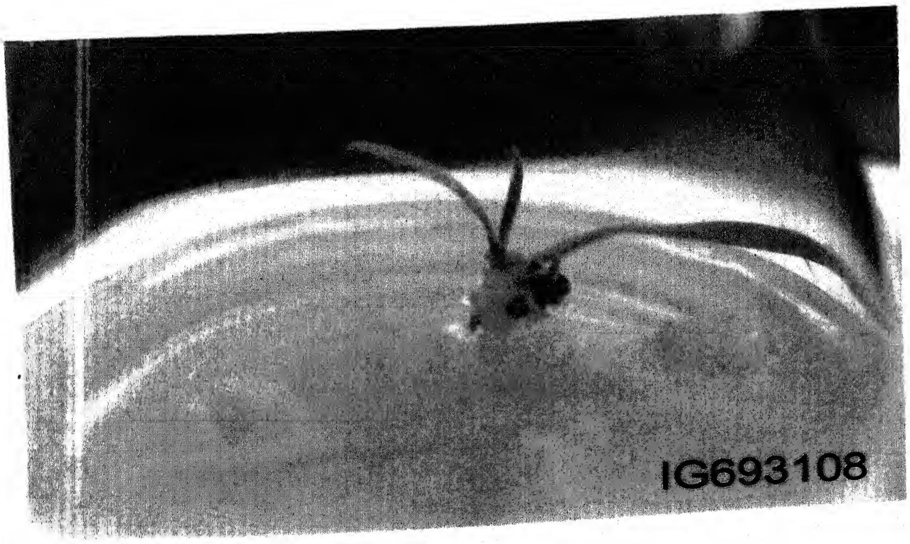


fig-3 Shoot induction

Plate # 9

Regeneration

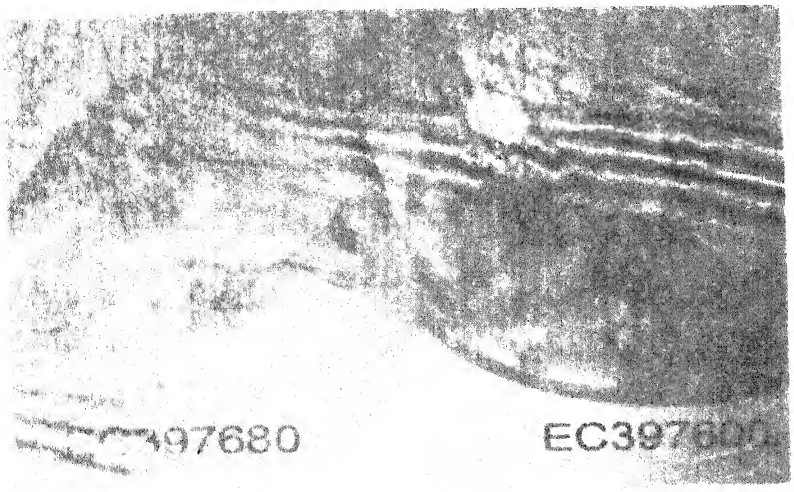


fig-1 Shoot induction

fig-2 Shoot induction *C. setigerus*

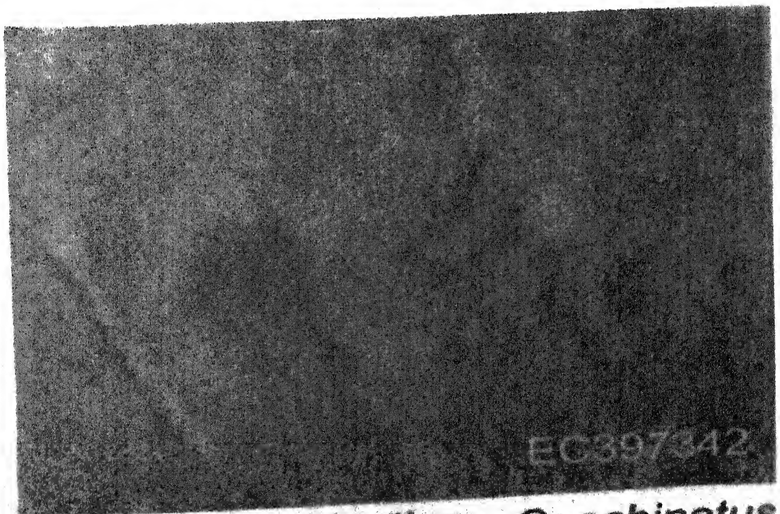


fig-3 Shoot induction *C. echinatus*

Plate # 10

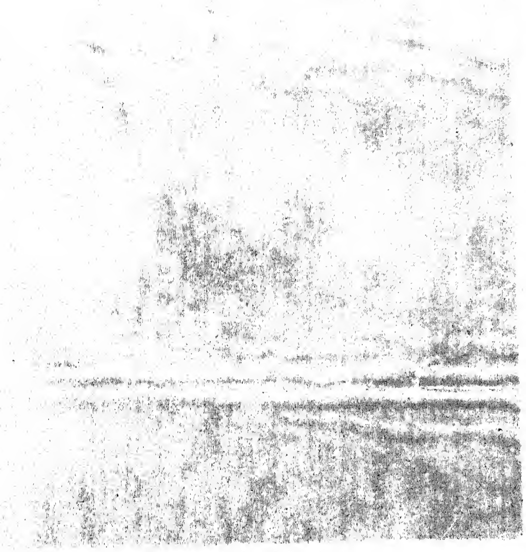


Fig-1 *C.ciliaris*

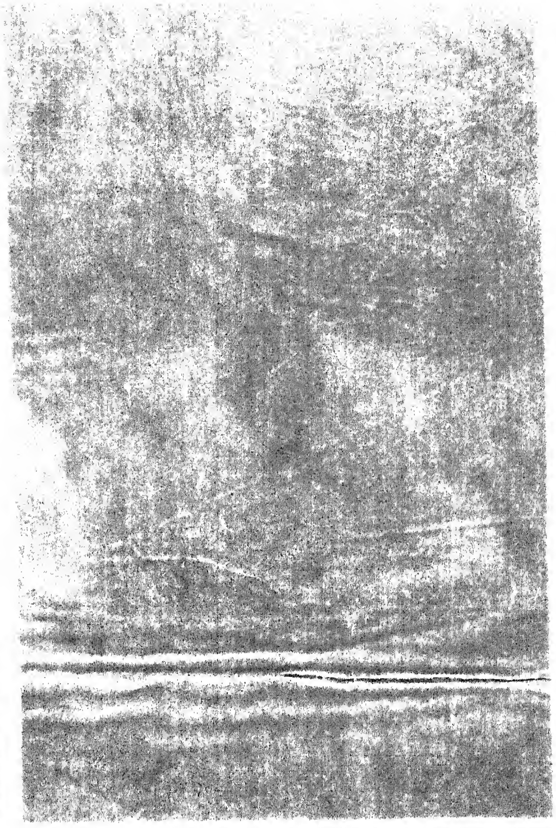


Fig-2 *C.setigerus*

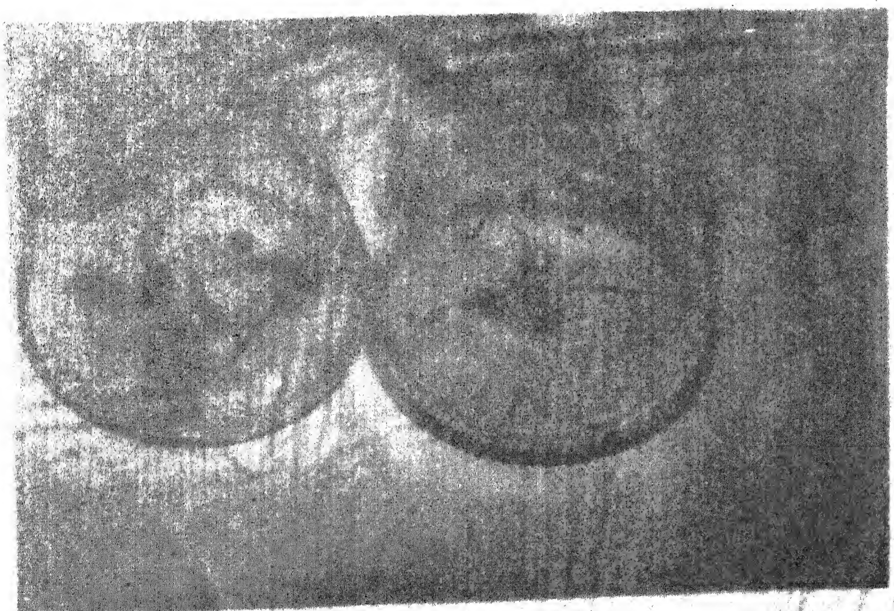


Fig-3 *C.echinatus*

Plate # 11

Histological observation

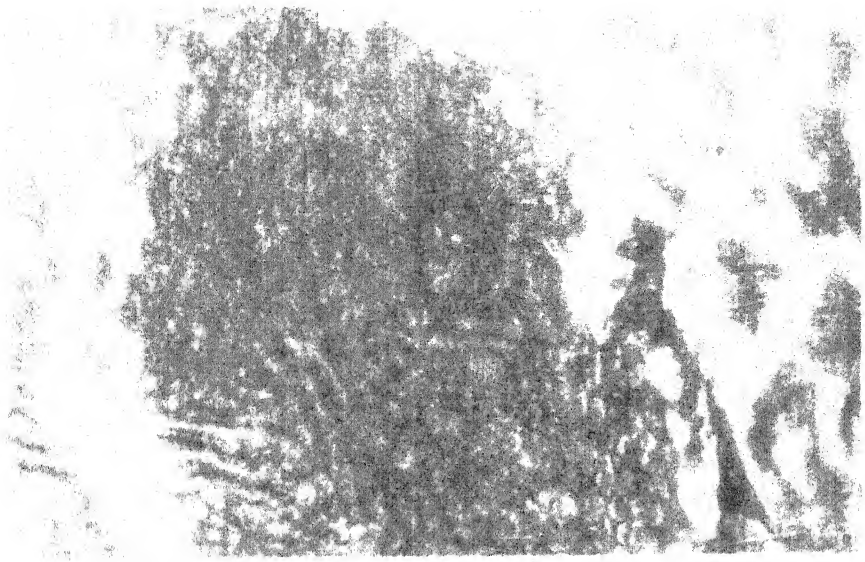


fig-1 Regenerating callus

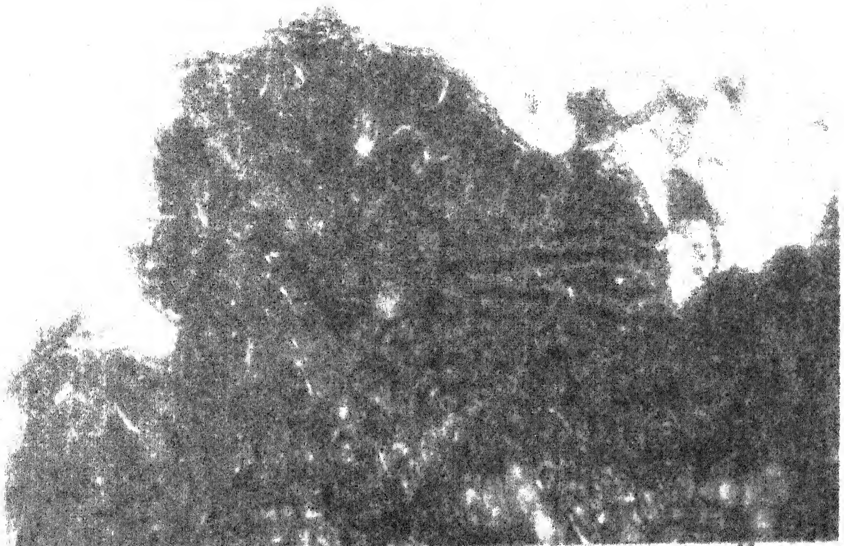


fig-2 Starch granule containing cell

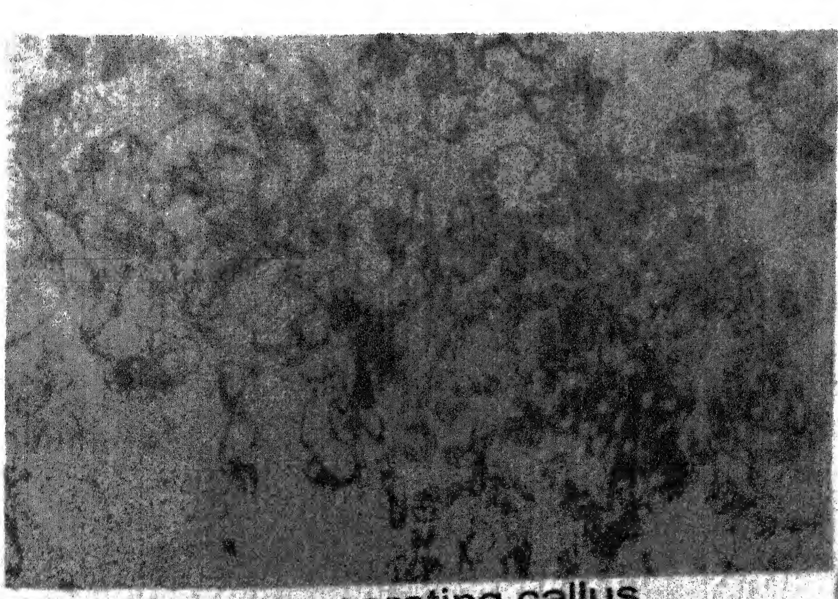


fig-3 Non-regenerating callus

Plate # 12

[illegible]

fig-1 Shoot bud organogenesis

fig-2 Multiple shoot buds

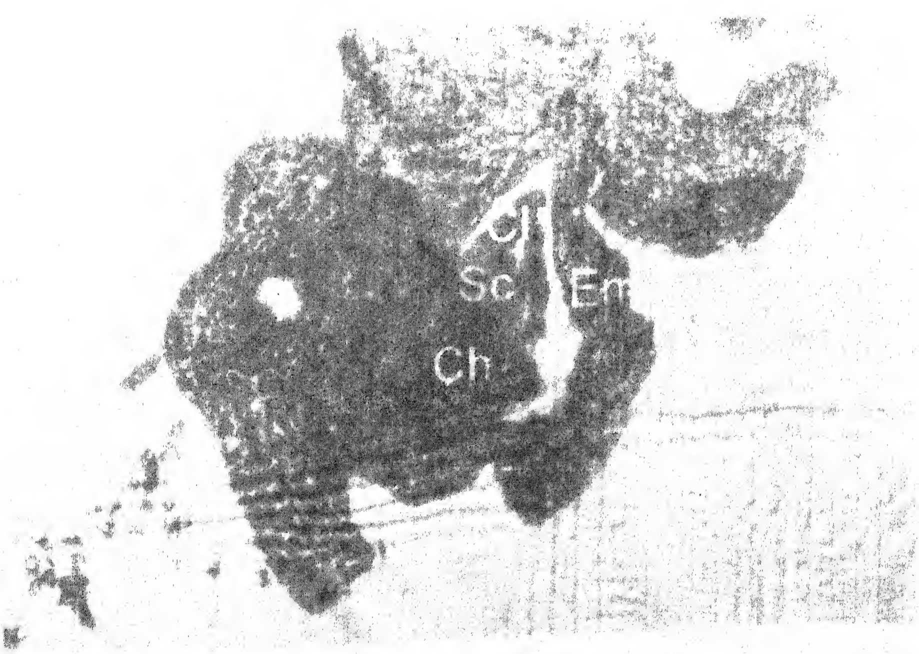


fig-3 Somatic embryogenesis

Em - Somatic embryo, Ch- colerorrhiza
Cl - coleoptile, Lp - leafprimordia
Sa - shoot apex

media containing 2.0 mg/l kin alone (0.84) (plate # 9, fig,3). No other media was comparable to this medium for regeneration in this species.

All other media combinations for regeneration showed regenerating frequency of 0.24 shoots/100 mg of callus or less which were much lower than the performance of media containing 2.0 mg/l kin alone. Addition of 0.2 mg/l 2,4-D in the media led to reduction in regeneration frequency, the lowest being 0.16 shoots/100 mg of callus which was observed in the media containing 2.0 mg/l kin. There was also a decrease in regeneration frequency with the other cytokinin (BA) when supplemented with 0.2 mg/l 2,4-D upto the minimum of 0.12 shoots/100 mg of callus. Whereas, without the application of 2,4-D, it showed better regeneration (0.18 shoots/100 mg of callus).

The least response for regeneration potential (0.09 shoots/100 mg of callus) was observed on the media contained 5.0 mg/l kin+0.2 mg/l 2,4-D. However, multiple shoot bud formation in addition to shoot bud organogenesis was also observed on regeneration medium with a composition of MS basal+2.0 mg/l kin alone (plate # 10, fig.3).

(C) Rhizogenesis

(i) *C.ciliaris:*

Most of the regenerated shoots were devoid of roots, though rarely few shoots had developed some thin fibrous root in some prolonged cultures of shoot regeneration. This suggested for the requirement to transfer shoots to root induction medium for proper root development and plantlet formation. Experiment on rhizogenesis was carried after shoot formation. Eight different types of media were used for root induction. The basal media used were half MS basal and half MS basal plus indol butyric acid (IBA) (table 4.26). The regenerating shoots (1.0 to 1.5 cm long) growing in cultures were isolated along with a bit of surrounding callus and transferred aseptically on the rooting media. After a week of transfer to the rooting medium the roots started developing and within three weeks' time plantlets with well developed roots were formed.

Genotypes exhibited nonsignificant difference among them for root differentiation (table 4.26). Maximum rooting response was noticed in

genotypes EC400587 and IG693108 with 4.75 roots/shoot (plate # 13, fig. 2), which was at par with EC400631 (4.31) (Plate # 13, fig. 1) and followed by EC400610 (4.06) (Plate # 13, fig. 3). The genotype EC397680 (Plate # 14, fig. 1) with 3.63 roots/shoot and EC397600 (Plate # 13, fig. 3) with 3.62 roots/shoot were the poorest responders among all the *C.ciliaris* genotypes.

The media had highly significant difference among them. Highest rooting performance for roots/shoot was noticed on 0.5 mg/l IBA+2 g/l charcoal containing media (8.60) followed by 10.0 mg/l IBA+2 g/l charcoal (5.08) and 1.0 mg/l IBA containing media (5.0). The media with 1.0 mg/l IBA+0.2 mg/l BA, 2.0 mg/l IBA+2 g/l charcoal and 0.5 mg/l IBA were comparable for rooting efficiency exhibiting 3.91, 3.5 and 3.16 roots/shoot, respectively. The minimum number of roots/shoot were counted on the media containing 0.5 mg/l IBA+0.2 mg/l BA (1.41) followed by the half strengthen of MS medium alone (2.5).

Interaction between media and genotypes was highly significant. The highest rooting response 12.5 roots/shoot occurred in the genotype IG693108 on 0.5 mg/l IBA+2 g/l charcoal and the minimum rooting response 0.5 roots/shoot was represented by the genotype EC397680 on half MS alone and by EC397600 on 0.5 mg/l IBA containing media. Increase in the IBA concentration led to gradual decrease in root induction. Media 0.5 mg/l IBA+2 g/l charcoal showed maximum on root induction capacity (Plate # 14, fig. 4) in all the genotypes tried except in EC400631.

(ii) *C.setigerus*

With respect to root formation in shoot cultures of *C.setigerus*, no significant difference was found among media (table 4.27). Highest performing media contained 0.5 mg/l IBA+2 g/l charcoal with root induction effect of 6.5 roots/shoot. The medium with 1.0 mg/l IBA+2 g/l charcoal was found to be similar in response (4.5 roots/shoot). The half MS basal medium and the medium with 2.0 mg/l IBA+2 g/l charcoal also performed similarly (3.5 roots/shoot) for root induction. The minimum number of roots were produced on the media containing 0.5 mg/l IBA+0.2 g/l BA (1.5 roots/shoot) (Plate # 14, fig. 2).

Table 4.26: Effect of media on-root induction frequency (no. of roots/shoot) from shoot cultures of the six genotypes of *C.ciliaris*.

Media	Genotype						
MS	EC397587	EC397680	EC400637	EC400600	EC400610	IG693108	Mean
½ strength	2.50	0.50	4.50	1.50	2.50	3.50	2.50
0.5 IBA	0.50	2.00	3.50	2.00	3.50	7.50	3.16
1.0 IBA	4.50	4.00	6.50	5.50	3.00	6.50	5.00
0.5 IBA+2g charcoal	9.50	8.50	1.50	11.00	9.00	12.50	8.66
1.0 IBA+2g charcoal	9.00	4.00	8.00	2.50	5.50	1.50	5.08
2.0 IBA+2g charcoal	6.00	3.00	3.00	1.50	3.50	4.00	3.50
0.5 IBA+0.2 BA	1.00	2.00	1.50	2.00	1.50	0.50	1.41
1.0 IBA+0.2 BA	5.00	3.50	6.00	3.00	4.00	2.00	3.91
Mean	4.75	3.43	4.31	3.62	4.06	4.75	

F test
Genotype 1.01
Media 11.85**
Interaction 2.07**

Table 4.27: Root induction efficiency in *C.setigerus*

Media MS	Number of roots per shoot
½ MS	3.5
0.5 IBA	4.0
1.0 IBA	4.5
0.5 IBA+2g ch	6.5
1.0 IBA+2g ch	4.5
2.0 IBA+2g ch	3.5
0.5 IBA+2g BA	1.5
1.0 IBA+2g BA	3
Mean	3.87
F test	1.05

Table 4.28: Effect of different media combinations on root induction efficiency in *C.echinatus*

Media MS	Number of roots per shoot
½ MS	2.5
0.5 IBA	2.5
1.0 IBA	4.0
0.5 IBA+2g ch	3.5
1.0 IBA+2g ch	4.0
2.0 IBA+2g ch	2
0.5 IBA+2g BA	1.5
1.0 IBA+2g BA	2.5
Mean	2.81
F test	0.41

Plate # 13

Rhizogenesis in *C.ciliaris*

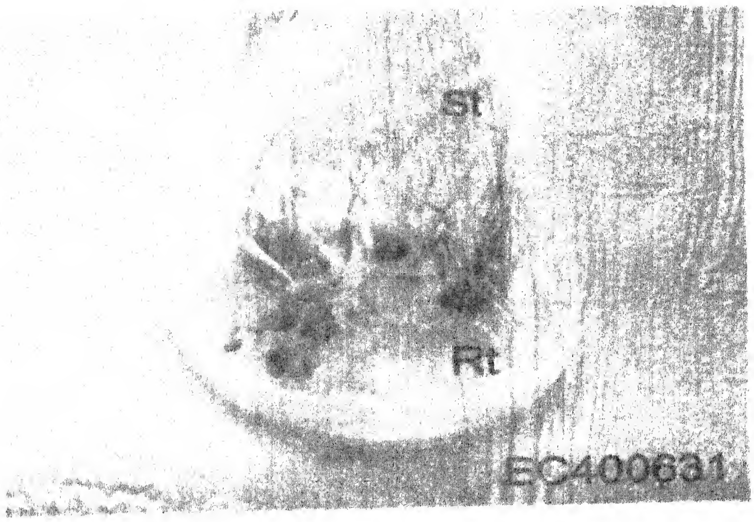


fig-1

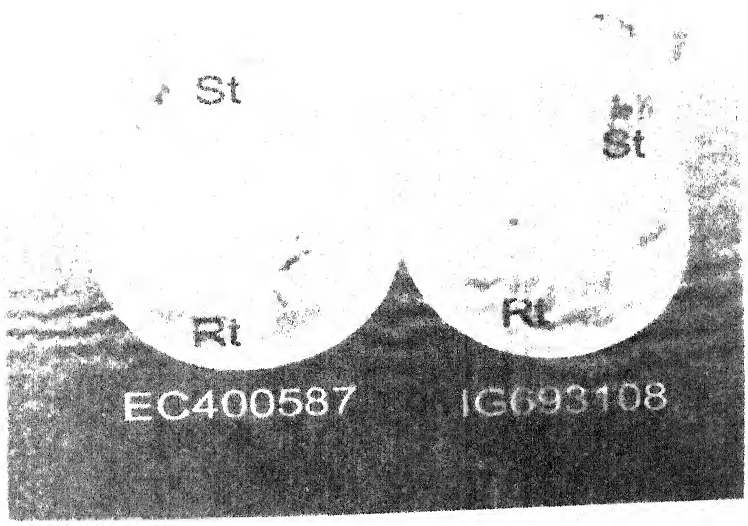


fig-2

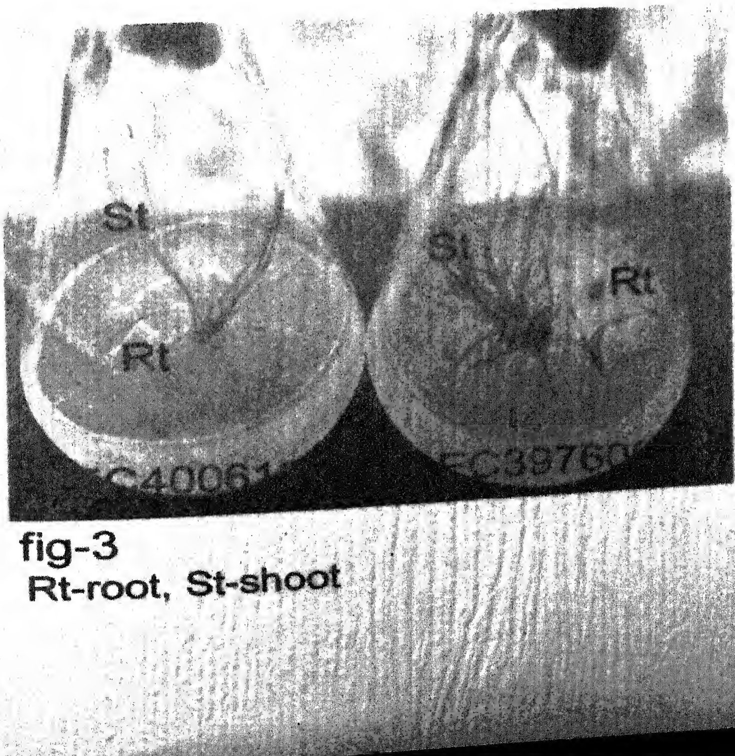


fig-3

Rt-root, St-shoot

Plate # 14

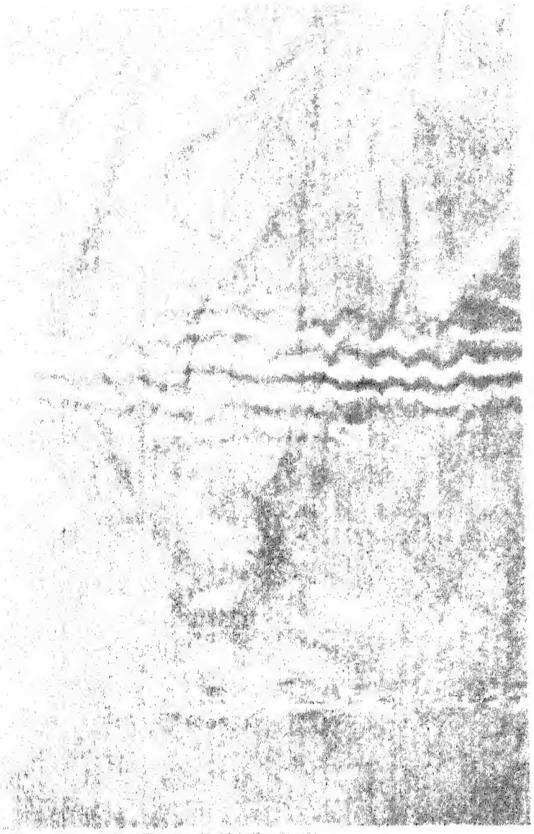


fig-1 EC397680
(*C. ciliaris*)

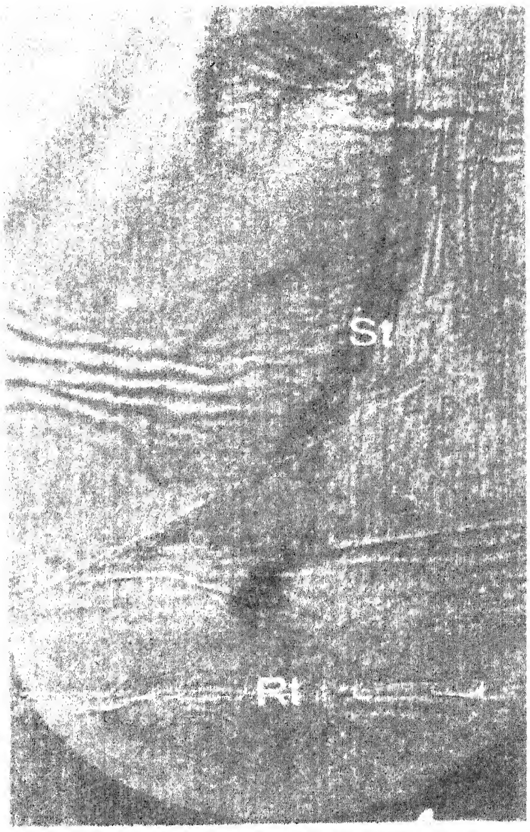


fig-2 EC400639
(*C.setigerus*)

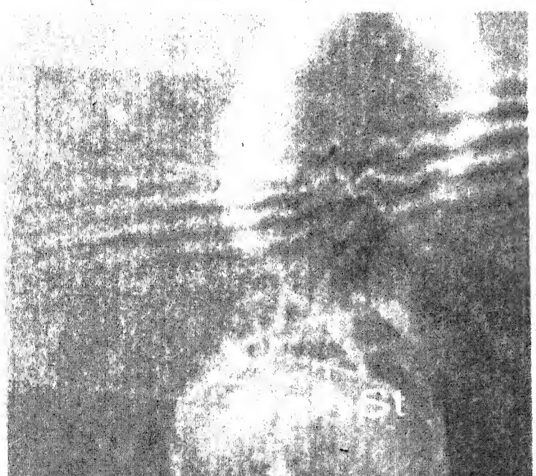


fig-3 EC397342
(*C. echinatus*)

fig-4
(*C. ciliaris*)

CM-Medium with charcoal
Rt-root, St-shoot

(iii) *C. echinatus*

The effect of different media combinations for root induction response on the *in vitro* regenerated shoots in *C. echinatus* is presented in Table – 4.28. The media had no significant effect for root formation in *C. echinatus* (EC397342). On an average, it showed 2.81 roots/shoot. Maximum rooting efficiency in terms of roots/shoot was noticed on the medium supplemented with 1.0 mg/l IBA+2 g/l charcoal (4.0) and 1.0 mg/l IBA alone (4.0) (Plate # 14, Fig. 3) followed by 0.5 mg/l IBA+2 g/l charcoal (3.5). The half MS basal medium alone and the media containing 0.5 mg/l IBA and 1.0 mg/l IBA+0.2 mg/l BA were similar in performance for root induction, exhibiting 2.5 roots/shoot. The Minimum rooting response was found with the media containing 2.0 mg/l IBA+2 g/l charcoal (2.0 roots/shoot) and 1.0 mg/l IBA+0.2 mg/l BA (2.5 roots/shoot).

4.1.4 Hardening and acclimatization of plantlets

The plantlets with well-developed shoot and roots were transferred on semisolid agar medium containing 0.4% sucrose prepared with ordinary (tap) water and kept controlled culture conditions at 25±2^{0}C and 16 hours light/8 hours dark photoperiod for 20-21 days. These plantlets were taken out of the culture flasks and their roots were washed properly with sterile water to remove the adhering medium. These plantlets were then placed in the test tubes having sterile tap water for 2-3 days in such a way that the roots were almost moist (Plate # 15, fig. 1). These plants were subsequently transferred to the pots containing soil, sand and FYM (1:1:1 v/v) and kept in culture conditions for 2-3 days and then transferred to the diffused light conditions at room temperature for 2-3 days (Plate # 15, Fig. 2). These pots containing cultured plants were transferred to field conditions and kept for seven days and then planted in the experimental plots in the field (Plate # 16, Fig. 1). All the *in vitro* regenerated plants transferred to the field conditions did not survive in the field conditions. The survival of these plants was also genotype specific but the plants once established in the field, survived to maturity (Plate # 16, fig. 2). Table 4.29 indicates the percentage survival of hardened plantlets of *C. setigerus*, *C. echinatus* and the different genotypes of *C. ciliaris*, regenerated *via*

Table 4.29: Percentage survival of plantlets from different explants of *Cenchrus* species through *in vitro* culture

Genotype	Number of regenerated plantlets	Number of survived plantlets	Percentage (%) survival in pots	Number of somaclones survived in field	Percentage (%) survival in field
EC400631	44	28	63.6	16	57
EC397600	9	3	33.3	2	66.6
EC400587	2	1	50	1	100
IG693108	19	10	52.6	5	50
EC400610	44	22	50	7	31.8
EC397680	14	6	54.3	2	33.33
C.setigerus (EC400639)	20	20	100	17	85
C.echinatus (EC397342)	71	43	60.5	25	58

Plate # 15

Hardening and Acclamatization

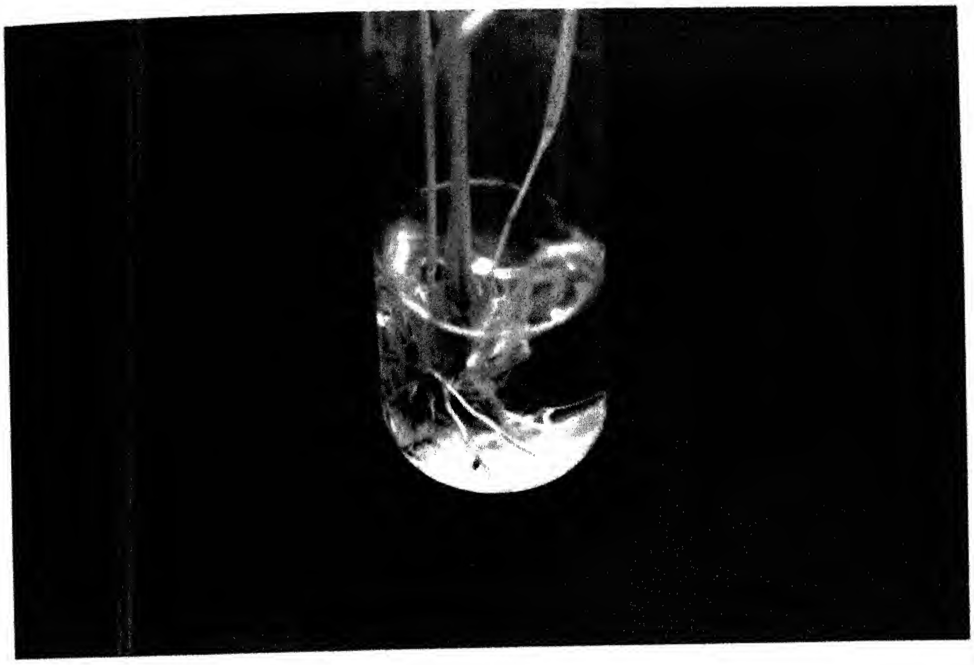


fig-1 Plantlets in tab water

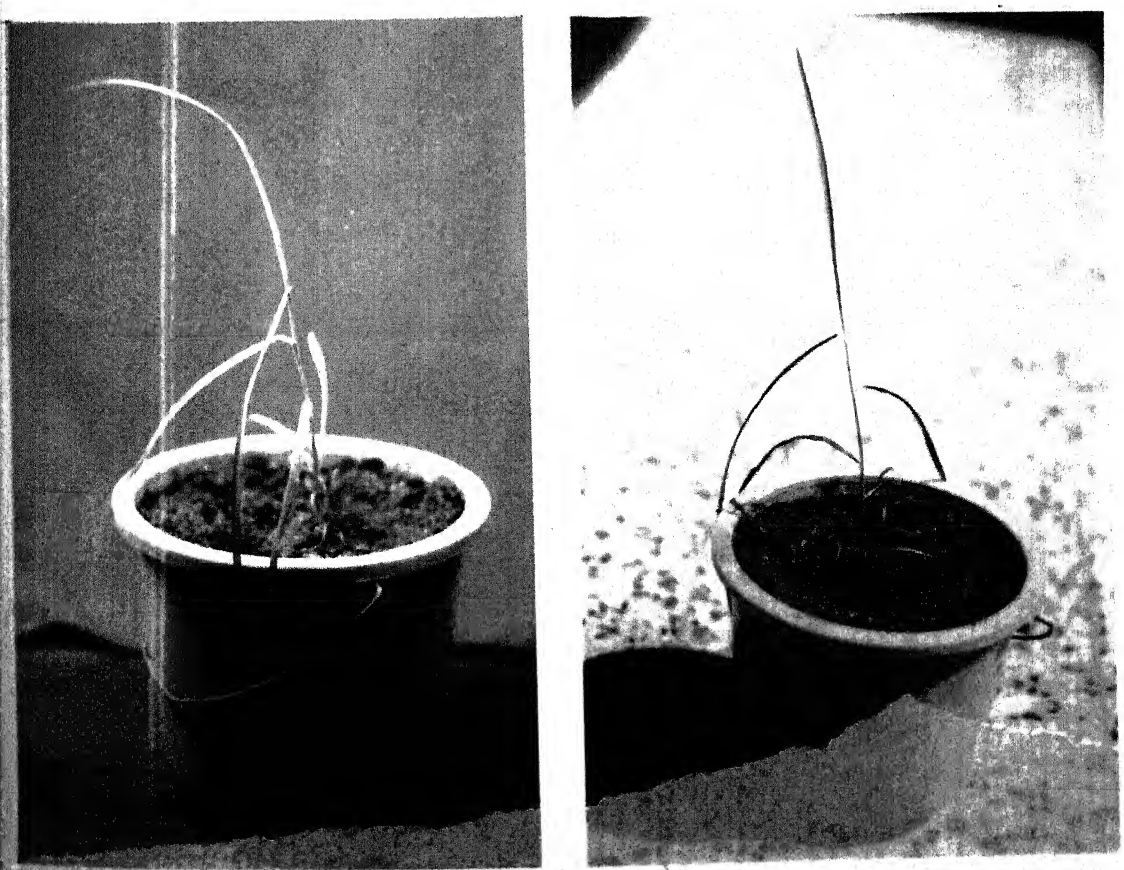


fig-2 Plantlets in pot

Plate # 16

Somaclones in the field

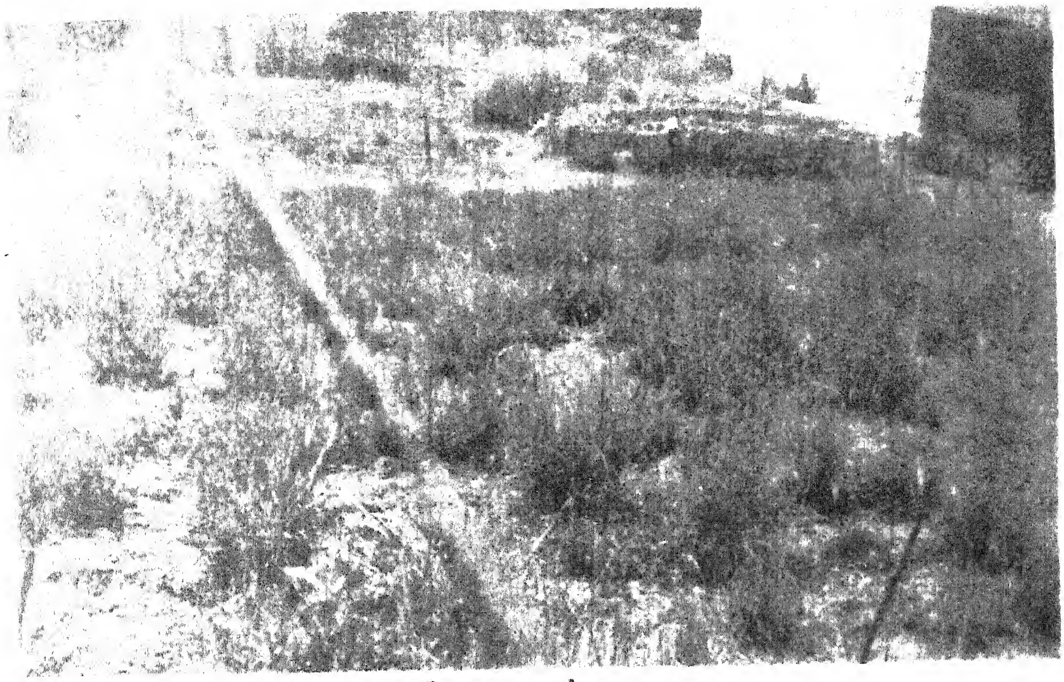


fig-1 Early stage of growth

fig-2 Somaclones at maturity

in vitro culture. Plant survival varied from species to species and genotype to genotype. In *C.setigerus*, out of 20 plantlets subjected to hardening all survived during hardening process but from pots to the field only 17 (85%) plants could survive and reached to maturity. In *C.echinatus*, out of 71 plantlets placed for hardening, only 43 (60.5%) plants could survive in pots and out of these 43 plants only 25 plant (58%) remained viable in the field conditions and developed upto maturity.

Percentage survival of plantlet of *C.ciliaris* showed genotypic difference. Least survival was found in the genotype EC397600 as out of 9 plantlets, only 3 (33.33%) were hardened and out of these three only two plantlets survived in the experimental plot. While the highest survival rate of the plantlets was observed in the genotype EC400631 where 28 plants could withstand hardening out of 44 plantlets (63.6%) and from these 28 plants only 16 survived (57%) in field conditions. Remaining genotypes gave intermediate response. In genotype IG693108, 10 plants out of 19 plantlets (52.6%) survived during hardening and 5 plants from these 10 hardened plants (50%) remained viable in field. In the genotype EC397680, 54.3% plantlets (6 plants out 11 plantlet survived during hardening) and out of these surviving plants only two (33.3%) survived in the field. The survival of plantlets during hardening was 50% in the genotypes EC400680 (6 plants from 11 plantlets) and EC400587 (1 plant from two plantlets). In EC400610 only two plants out of 6 hardened plants (33.33%) could survive in the field whereas only one plant of EC400587 which survived through hardening also survived when transferred from pot to field conditions and developed to maturity.

4.2 Evaluation of somaclones and their progenies and the comparison with respective oparent material for somaclonal variation

4.2.1 Morphological observation

The *in vitro* regenerated plants from six genotypes of *C.ciliaris* and from one each genotype of *C.setigerus* and *C.echinatus* were planted in the field after hardening.

Observiations were recorded on all plants for morphological variations and biometrical characters of agronomic importance.

4.2.1.1 Quantitative characters

(A) Variation in somaclones

(a) *C.ciliaris*

Evaluation of variation in somaclones (derived from seed explants) of the genotype EC400631 is presented in table 4.30. Uni-and bi-directional variations were recorded for all quantitative traits in the seed explant derived somaclones as indicated by a wider range and higher standard deviation (SD) and coefficient of variation (CV) (up to 57%) (plate # 17, fig.1). The average leaf length, leaf width, internodal length, spike length, spike width, number of spikelets per spike 100 bur weight and days to flowering increased in comparison to the parent material. The variation for spikelets spike, peduncle length and internodal length increased greatly in the somaclones during kharif 1999.

During kharif 2000, somaclones exceeded over the control for number of tillers, leaf width, spike width, spikelets/spike, 100 bur weight, green fodder yield (GFY) and days to flowering. In this season, also, bi-directional variations were recorded for all the traits except for plant height, leaf length, internodal length and peduncle length where somaclones performed poorly and for number of tillers per plant, spike length, spike width, spikelets/spike and 100 bur weight where all somaclones recorded higher values than the respective parents. During this season, greater variability was exhibited for DMY, GFY and 100 bur weight (table 4.30).

Evaluation of somaclones of the same genotype derived from immature inflorescence explant indicated enhanced variation with higher SD and CV (upto 40.1%) (table 4.31). Increased values for the number of tillers, leaf length, internodal length, GFY and DMY were observed and the variation for GFY, leaf length, number of tillers, number of spikelets/spike and DMY increased greatly in the somaclones during the year 1999.

Table 4.30: Somaclonal variation for quantitative traits in the seed explant derived somaclones of the genotype, EC400631.

	KHARIF 1999						KHARIF 2000					
CHARACTER	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	84.00	101.00	92.50	90.08	12.02	13.00	56.0	72.0	64.00	56.33	11.31	17.68
Plant Height (cm)	63.50	70.20	66.85	79.91	4.74	7.09	103.0	112.0	107.50	126.2	6.63	5.92
Number of tillers	5.00	6.00	5.50	8.17	0.71	12.86	18.0	23.0	20.50	17.42	3.54	17.25
Number of leaves per tiller	8.00	10.00	9.00	11.08	1.41	15.71	12.0	16.0	14.00	15.08	2.83	20.20
Leaf length (cm)	13.40	17.96	15.68	13.66	3.22	20.56	25.23	25.76	25.49	31.25	0.37	1.47
Leaf width (cm)	0.60	0.70	0.65	0.55	0.07	10.88	0.66	0.80	0.73	0.63	0.10	13.56
Internodal length (cm)	3.20	5.60	4.40	4.36	1.70	38.37	6.20	7.30	6.75	7.79	0.78	11.52
Number of spikelets per spike	42.0	99.00	70.50	65.70	40.31	57.17	86.33	100.3	93.32	57.50	9.88	10.59
Peduncle length (cm)	11.50	23.20	17.35	20.02	8.27	47.68	11.60	14.20	12.90	17.40	1.84	14.25
Spike length (cm)	7.30	8.03	7.66	7.24	0.52	6.73	8.90	8.96	8.93	7.32	0.04	0.47
Spike width (cm)	1.20	1.33	1.27	0.90	0.09	7.27	1.00	1.43	1.21	0.80	0.30	25.03
100 bur weight (gm)	0.29	0.40	0.34	0.25	0.08	22.25	0.21	0.41	0.31	0.17	0.14	45.62
Green fodder yield (gm)	40.0	50.0	45.00	46.67	7.07	15.71	100.0	220.0	160.0	115.83	84.85	53.03
Dry matter yield (gm)	12.0	15.70	13.85	18.52	2.62	18.89	15.10	76.50	45.80	46.55	43.42	94.80

Table 4.31: Somaclonal variation for quantitative traits in the immature inflorescence explant derived somaclones of the genotype, EC400631.

CHARACTER	KHARIF 1999						KHARIF 2000					
	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	80.0	108.0	85.83	90.08	6.25	7.28	26.0	79.0	61.0	56.33	17.84	29.24
Plant Height (cm)	53.5	131.50	83.36	79.91	18.54	22.25	91.0	142.50	118.14	126.20	12.54	10.62
Number of tillers	6.00	19.00	11.94	8.17	4.05	33.91	12.00	57.00	36.22	17.42	14.46	39.91
Number of leaves per tiller	8.00	15.00	11.00	11.08	2.25	20.45	9.00	15.00	11.94	15.08	1.47	12.34
Leaf length (cm)	9.66	28.03	19.01	13.66	6.20	32.63	26.06	43.60	34.37	31.25	5.22	15.20
Leaf width (cm)	0.30	0.63	0.47	0.55	0.09	22.01	0.50	0.70	0.57	0.63	0.07	12.16
Internodal length (cm)	2.50	9.30	5.28	4.36	1.38	26.11	4.50	10.20	7.11	7.79	1.48	20.87
Number of spikelets per spike	22.0	84.0	62.09	65.70	18.18	29.28	86.33	100.30	93.32	57.50	9.88	10.59
Peduncle length (cm)	10.40	29.50	21.20	20.02	5.35	25.22	11.50	23.40	16.83	17.40	4.14	24.54
Spike length (cm)	3.55	9.20	6.47	7.24	1.53	23.71	6.40	9.16	7.90	7.32	0.90	11.36
Spike width (cm)	0.55	1.30	0.90	0.90	0.22	25.03	0.80	1.26	1.03	0.80	0.14	13.76
100 bur weight (gm)	0.08	0.23	0.15	0.25	0.03	23.78	0.08	0.18	0.12	0.17	0.03	25.93
Green fodder yield (gm)	40.0	195.0	87.78	46.67	35.20	40.10	50.00	770.00	261.11	115.83	198.08	75.86
Dry matter yield (gm)	17.50	45.60	30.19	18.52	8.82	29.23	12.00	219.56	76.35	46.55	53.86	70.55

Table 4.32: Somaclonal variation for quantitative traits of the genotype, EC397600.

	KHARIF 1999						KHARIF 2000					
CHARACTER	Mini-mum	Maxi-mum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Mini-mum	Maxi-mum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	76.0	94.0	85.0	89.67	12.73	14.97	28.00	43.00	35.50	44.67	10.61	29.88
Plant Height (cm)	80.50	85.50	83.0	83.30	3.54	4.26	79.50	103.15	91.36	103.73	16.69	18.28
Number of tillers	7.0	8.0	7.50	14.33	0.71	9.43	15.00	25.00	20.00	50.67	7.07	35.36
Number of leaves per tiller	6.0	7.0	6.50	7.0	0.71	10.88	10.00	12.00	11.00	10.33	1.41	12.86
Leaf length (cm)	22.96	23.66	23.31	32.92	0.49	2.12	35.83	44.73	40.28	41.45	6.29	15.62
Leaf width (cm)	0.63	0.73	0.68	0.72	0.07	10.40	0.50	0.60	0.55	0.69	0.07	12.86
Internodal length (cm)	7.30	8.60	7.95	7.87	0.92	11.56	3.50	7.10	5.30	4.00	2.55	48.03
Number of spike-lets per spike	74.0	86.66	80.33	89.55	8.95	11.14	9.66	9.66	7.66	41.44	-	-
Peduncle length (cm)	19.60	22.40	21.00	18.47	1.98	8.43	16.50	16.50	16.50	23.53	-	-
Spike length (cm)	7.73	8.53	8.13	9.26	0.57	6.96	2.00	2.00	2.00	4.44	-	-
Spike width (cm)	1.00	1.30	1.15	1.21	0.21	18.45	0.90	0.90	0.90	0.91	-	-
100 bur weight (gm)	0.52	0.81	0.66	0.62	0.21	30.84	0.64	0.64	0.64	0.33	-	-
Green fodder yield (gm)	55.0	90.0	72.50	223.33	24.75	34.14	80.00	420.00	250.00	240.00	240.42	96.17
Dry matter yield (gm)	13.70	15.80	14.75	46.53	1.48	10.07	67.50	110.30	88.89	57.47	30.26	34.04

Table 4.33: Somaclonal variation for quantitative traits in the genotype, IG693T08

	KHARIF 1999						KHARIF 2000					
CHARACTER	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	83.0	96.0	95.6	89.0	5.43	6.10	52.00	70.00	74.50	62.40	9.13	14.63
Plant Height (cm)	62.50	127.50	89.43	95.14	23.86	25.08	100.00	155.00	153.97	124.67	24.60	19.73
Number of tillers	2.0	16.0	11.18	9.60	5.13	53.42	20.00	91.00	31.00	52.83	27.82	52.66
Number of leaves per tiller	9.0	13.0	10.45	10.0	1.73	17.32	9.00	15.00	13.73	12.50	2.43	19.43
Leaf length (cm)	2.83	33.50	20.32	19.24	12.96	67.24	23.96	42.06	51.25	31.52	7.80	24.76
Leaf width (cm)	0.30	0.96	0.54	0.69	0.25	39.13	0.50	0.70	0.61	0.56	0.08	15.03
Internodal length (cm)	2.90	9.30	5.18	6.56	2.93	44.62	4.50	8.50	9.14	6.55	1.27	19.33
Number of spike-lets per spike	32.66	131.00	73.19	69.73	36.94	52.94	70.66	243.66	131.67	118.58	83.55	70.46
Peduncle length (cm)	7.80	21.70	26.10	16.34	5.74	35.12	18.40	27.20	22.00	21.33	4.01	18.81
Spike length (cm)	3.46	9.16	7.65	6.59	2.48	37.59	5.13	10.66	8.72	6.71	2.64	39.32
Spike width (cm)	0.73	1.30	0.80	1.02	0.26	25.77	1.06	1.36	1.04	1.17	0.13	11.29
100 bur weight (gm)	0.19	0.32	0.18	0.26	0.07	26.83	0.16	0.25	0.13	0.20	0.04	19.91
Green fodder yield (gm)	8.00	360.00	91.48	145.60	134.41	92.32	130.00	620.00	170.32	351.67	248.63	70.7
Dry matter yield (gm)	4.60	105.10	34.69	42.94	38.74	90.22	51.67	208.90	73.71	102.24	59.42	58.12

Characterization of somaclones during the year 2000 revealed bi-directional variations for all the quantitative traits except number of leaves per tiller, spike width and number of spikelets/spike (table 4.31). Somaclones were found better than their parents for the number of tillers, leaf length, spike length, spike width, number of spikelet/spike, GFY and DMY. Greater variations in somaclones were shown by GFY and DMY during this season.

Increased bi-directional variations in 1999 were found for all the traits except number of tillers, leaf length, spike length, number of spikelets/spike, GFY and DMY where all the somaclones of genotype EC397600 decreased greatly (table 4.32). Internodal length, peduncle length and 100 bur weight increased greatly in somaclones. Greater variation in somaclone was shown by 100 weight and GFY during this season.

Increased bi-directional variations were also recorded during year 2000 (table 4.32), except for days to fowering, plant height, number of tillers, leaf width, spike length, spike width, peduncle length, and spikelets/spike where all somaclones recorded decreased value but hundred bur weight where somaclones exhibited increment. Wide range of variation was shown by the somaclones as indicated by higher SD and CV (up to 96%). Internodal length, GFY, number of leaves per tiller, and DMY increased. However, days to flowering and plant height decreased. Greater variability was recorded for all the traits except number of leaves per tiller, leaf width and leaf length.

Increased bi-directional variations except for peduncle length and 100 bur weight were observed for all traits in the somaclones of IG693108 during the kharif 1999 (table 4.33) as indicated by wider range, SD and CV (upto 92.4%). Plant height, leaf width, internodal length, spike width and hundred bur weight increased in somaclones. Variation for number of tillers, leaf length, GFY, DMY, number of spikelets/spike, Internodal length and leaf width found to be increased greatly.

During the year 2000, enhanced bi-directional variations were also observed for all the quantitative traits except leaf length, where somaclones exhibited poor performance and hundred bur weight where somaclones showed better performance than parent. Great increase in GFY and DMY and reduction in

Table 4.34: Somaclonal variation for quantitative traits in the genotype, EC400610.

CHARACTER	KHARIF 1999						KHARIF 2000					
	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	84.00	91.00	112.00	87.50	4.95	5.66	31.00	85.00	41.00	45.14	19.13	42.39
Plant Height (cm)	79.80	131.20	58.53	105.5	36.35	34.45	51.10	160.00	104.40	85.25	36.53	42.85
Number of tillers	3.00	25.00	9.67	17.00	12.73	74.87	12.00	146.00	83.71	49.88	42.19	84.60
Number of leaves per tiller	4.00	9.00	7.00	6.50	1.51	23.26	5.00	13.00	10.00	9.88	3.04	30.83
Leaf length (cm)	5.03	28.80	18.37	15.60	8.19	52.48	20.50	36.4	31.76	28.73	4.87	16.96
Leaf width (cm)	0.40	0.63	0.51	0.47	0.09	18.79	0.50	0.70	0.61	0.56	0.08	15.03
Internodal length (cm)	1.80	22.40	3.07	5.79	6.80	117.51	1.50	11.50	4.19	4.41	3.11	70.18
Number of spikelets per spike	19.00	67.66	42.26	41.66	16.37	39.29	42.00	154.33	181.38	96.57	42.60	54.47
Peduncle length (cm)	5.20	22.50	10.37	12.54	4.90	39.10	7.50	26.50	20.49	19.46	6.33	32.53
Spike length (cm)	2.30	5.10	4.12	3.69	0.97	26.28	2.85	10.73	8.57	6.02	2.68	44.54
Spike width (cm)	0.56	1.03	0.66	0.82	0.19	23.10	0.70	1.00	1.02	0.84	0.11	13.59
100 bur weight (gm)	0.19	0.33	0.30	0.25	0.05	21.43	0.04	0.23	0.31	0.15	0.06	41.15
Green fodder yield (gm)	1.50	120.00	52.14	37.31	46.03	123.36	30.00	620.00	380.00	209.38	224.38	107.17
Dry matter yield (gm)	0.30	27.9	13.8	8.45	9.12	107.90	8.70	217.00	116.77	90.99	86.35	94.90

Table 4.35: Somaclonal variation for quantitative traits in the genotype, EC397680.

CHARACTER	KHARIF 1999						KHARIF 2000					
	Minimum	Maximum	Mean of Somaclone	Mean of Parent.	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	84.0	91.0	112.0	87.5	4.95	5.66	58.00	58.00	99.0	58.00	-	-
Plant Height (cm)	79.80	131.20	58.53	105.50	36.35	34.45	122.00	159.50	144.30	140.75	26.52	18.84
Number of tillers	8.0	26.0	9.67	17.0	12.73	74.87	70.00	82.00	52.00	76.00	8.49	11.16
Number of leaves per tiller	11.0	15.0	11.33	13.0	2.83	21.76	15.00	15.00	16.50	15.00	0.00	0.00
Leaf length (cm)	17.83	35.73	12.42	26.78	12.66	47.26	28.06	59.96	36.58	44.01	22.56	51.25
Leaf width (cm)	0.63	1.26	0.44	0.94	0.45	47.14	0.66	1.33	0.80	1.00	0.47	47.61
Internodal length (cm)	6.60	8.30	3.43	7.45	1.20	10.14	6.50	10.26	6.35	8.35	2.62	31.33
Number of spikelets per spike	90.66	143.33	53.54	117.00	37.24	31.83	194.66	194.66	175.16	194.66	-	-
Peduncle length (cm)	12.10	32.10	17.63	22.10	14.14	63.99	26.20	26.20	15.95	26.20	-	-
Spike length (cm)	6.53	11.90	5.53	9.22	3.80	41.21	9.06	9.06	7.22	9.06	-	-
Spike width (cm)	0.36	1.03	0.79	0.69	0.47	68.17	1.40	1.40	1.25	1.40	-	-
100 bur weight (gm)	0.22	0.28	0.17	0.25	0.64	16.97	0.25	0.25	0.15	0.25	-	-
Green fodder yield (gm)	70.00	170.00	91.00	120.00	21.21	38.57	660.00	895.00	512.50	777.50	166.17	21.37
Dry matter yield (gm)	28.20	94.00	40.97	61.10	53.60	95.54	200.30	223.24	137.40	212.02	15.87	7.48

days to flowering were observed in somaclones. Greater variation was noted for number of spikelets/spike, GFY, DMY, spike length and number of tillers (table 4.33).

Evaluation of somaclones of the genotype EC400610 exhibited increased bi-directional variations for all the traits (table 4.34) except plant height and days to flowering as indicated by wider range and higher SD and CV (upto 123.36%). Plant height, number of tillers internodal length, peduncle length and spike width were noted with increased value from the parent. Variation for GFY, DMY, internodal length, number of tillers and leaf length increased greatly in the somaclones during kharif 1999.

During the year 2000, somaclones exceeded the control for internodal length and days to flowering (table 4.34). In this season also bi-directional variations were observed for all the traits except number of spikelets/spike and hundred bur weight where all the somaclones recorded lower value than the parent. Greater variability was exhibited by GFY, DMY, number of tillers, internodal length, number of spikelets/spike, plant height, days to flowering and spike length.

All traits showed increased unidirectional variation in the somaclones of EC397680 for all the traits except number of tillers, number of leaves, peduncle length, spike width, GFY and DMY during kharif 1999 (table 4.35). Increased value for all the traits were observed except spike width and days to flowering. Variation for number of tillers, DMY, GFY, spike width, peduncle length, leaf width and plant height increased greatly in the somaclones in this season (plate # 17, fig. 3). During the year 2000, somaclones exceeded the control for all the traits except days to flowering, plant height and number of leaves per tiller. In this season also unidirectional variation was observed for all the traits except plant height, leaf length and leaf width. Greater variations were recorded for leaf length, leaf width, internodal length and GFY (table 4.35).

(b) *C.setigerus*

During year 1999, somaclones of *C.setigerus*, EC400639 exhibited wide range of variation (table 4.36). Increased bi-directional variations were observed for

Table 4.36: Somaclonal variation for the quantitative traits in the somaclones of *C.setigerus*.

	KHARIF 1999						KHARIF 2000					
CHARACTER	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	77.00	96.00	83.40	81.20	3.94	4.85	24.00	45.00	33.67	32.05	5.12	15.99
Plant Height (cm)	40.20	121.10	100.27	97.05	20.05	21.31	102.90	148.60	101.70	120.44	14.35	11.91
Number of tillers	3.00	54.00	10.70	14.00	6.10	43.60	6.00	74.00	22.90	33.15	18.53	55.91
Number of leaves per tiller	7.00	15.00	10.80	11.75	1.97	16.00	7.00	14.00	9.70	10.65	2.01	18.85
Leaf length (cm)	10.06	23.96	19.77	15.36	3.56	23.20	34.46	56.06	44.94	48.60	5.88	12.09
Leaf width (cm)	0.23	0.50	0.36	0.35	0.08	21.58	0.50	1.13	0.55	0.73	0.14	19.45
Internodal length (cm)	2.50	6.30	4.18	4.72	1.03	21.79	3.60	11.50	4.42	7.18	1.93	26.92
Number of spikelets per spike	77.33	165.66	80.96	111.48	24.56	22.03	33.00	173.00	121.50	122.50	35.89	29.28
Peduncle length (cm)	10.30	21.00	14.44	14.66	3.15	23.55	5.90	20.80	15.42	12.98	4.48	34.5
Spike length (cm)	7.13	10.63	7.44	8.94	0.87	9.78	3.30	10.90	8.05	8.65	1.71	19.77
Spike width (cm)	0.56	0.93	0.56	0.76	0.10	13.60	0.50	0.90	0.57	0.68	0.10	14.78
100 bur weight (gm)	0.29	1.02	0.38	0.64	0.21	32.90	0.11	0.50	0.29	0.11	0.10	22.94
Green fodder yield (gm)	3.20	250.00	63.00	113.66	75.44	66.38	30.00	440.0	75.00	156.00	104.10	66.73
Dry matter yield (gm)	1.60	94.50	27.21	43.80	27.62	63.06	9.75	96.86	20.19	42.27	24.97	59.09

Table 4.37: Somaclonal variation for quantitative traits in the somaclones of *C.echinatus*

	KHARIF 1999					
CHARACTER	Minimum	Maximum	Mean of Somaclone	Mean of Parent	Standard Deviation	Coefficient of Variation
Days to flowering	61.00	98.00	66.00	78.20	9.32	11.92
Plant Height (cm)	19.50	52.50	59.46	42.61	6.63	15.56
Number of tillers	3.00	11.00	7.00	5.96	2.11	35.42
Number of leaves per tiller	1.00	8.00	5.20	5.56	0.96	17.29
Leaf length (cm)	8.26	22.00	14.74	15.69	3.60	22.92
Leaf width (cm)	0.60	1.13	0.66	0.88	0.13	15.33
Internodal length (cm)	0.50	7.10	3.34	3.92	1.50	38.32
Number of spikelets per spike	12.00	35.50	25.60	19.81	5.14	25.93
Peduncle length (cm)	3.30	34.40	30.18	14.27	5.10	35.75
Spike length (cm)	3.66	6.83	6.04	5.55	0.83	15.04
Spike width (cm)	0.90	1.36	1.32	1.16	0.15	12.93
100 bur weight (gm)	1.20	3.20	3.42	2.22	0.53	23.97
Green fodder yield (gm)	10.0	200.0	107.00	74.60	50.04	67.08

all the traits except spike width. Somaclones exhibited higher SD and CV (upto 66.38%). Number of tillers, number of leaves, internodal length, spike length, spike width, number of spikelets, hundred bur weight, GFY and DMY increased greatly in somaclones. Number of tillers, GFY, DMY and hundred bur weight indicated greater variation in this season.

During 2000 kharif, bi-directional variations were also recorded for all traits except plant height. All somaclones recorded higher value than parent for all traits except days to flowering, peduncle length and hundred bur weight. During this season, greater variability was exhibited by number of tillers. GFY. DMY, peduncle length and spikelets/spike (table 4.36).

(c) *C.echinatus*

Being an annual nature of this species. the data of only one season could be recorded in the somaclones. Evaluation of somaclones of EC397342 during the year 1999, revealed increased bi-directional variation for all quantitative traits (table 4.37) except plant height, peduncle length and hundred bur weight. All characters showed wide range of variation. Somaclones exceeded the control for number of leaves. leaf length, leaf width and internodal length. Greater variability for GFY. leaf width, internodal length. spike width and number of tillers was recorded.

[B] Progeny analysis

(a) *C.ciliaris*

The morphological data of somaclones and their progenies of *C.ciliaris*. EC400631 has been presented in table 4.38. The performance of the progenies for various parameters differed from their respective somaclones in terms of increase or decrease in the value of different parameters. Very few progenies and their somaclones exhibited similar or near to similar values for a very limited number of parameters as indicated by the data. More number of somaclones exhibited better performance over to that their progenies with regard to plant height, number of leaves, leaf width. number of spikelet per spike. peduncle length, spike length and spike width while the number of progenies exceeded over to their respective somaclone parent in perfomance of the plant character such as number of tillers per plant. leaf length internodal

Table 4.38: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, EC400631 during the year 2000.

	Days to flowering			Plant Height			Number of tillers			No. of leaves / tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone Number	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %
Explant Seed																					
110100	84	95	7.44	70.2	57.0	23.6	6	4.6	15.7	8	8.0	17.7	13.4	20.11	20.8	0.60	0.66	7.4	3.2	4.80	8.8
110200	101	100.35	79.4	63.5	72.35	31.9	5	9.12	28.9	10	10.76	29.3	18.0	24.60	22.1	0.70	0.54	11.0	5.6	5.58	36.8
Immature Inflorescence																					
120100	85	85.0	-	70.5	85.26	-	7	12.0	-	10	10.0	-	19.1	23.70	-	0.56	0.56	-	5.4	6.30	-
120300	89	84.0	-	71.2	80.30	-	12	12.0	-	10	9.0	-	23.7	28.06	-	0.56	0.60	-	4.5	5.50	-
120500	85	91.5	0.77	77.2	67.70	32.4	9	13.0	54.4	10	8.5	25.0	25.5	27.38	0.26	0.50	0.50	-	5.3	4.80	20.6
120600	91	88.0	-	60.5	85.40	-	11	12.0	-	12	12.0	-	26.6	24.06	-	0.50	0.56	-	2.5	5.30	-
120700	88	82.67	0.70	76.5	81.17	3.1	7	16.67	33.1	9	10.0	20.0	23.5	30.76	1.8	0.53	0.60	-	4.5	5.17	11.8
120800	82	81.33	3.95	74.2	78.17	32.8	13	10.0	52.9	8	8.67	24.0	26.6	27.75	12.8	0.53	0.62	11.7	3.7	6.43	10.6
121200	82	84.3	2.80	108.0	99.21	14.4	13	19.80	39.8	15	10.5	12.9	15.6	14.66	11.5	0.40	0.35	15.3	4.6	5.58	13.3
121300	84	83.33	1.83	131.5	99.53	8.9	13	28.33	29.4	13	10.33	5.6	17.1	16.81	11.7	0.40	0.38	18.1	9.3	4.97	11.1
121400	90	88.9	4.16	114.5	83.31	21.4	13	15.40	34.9	14	11.7	10.7	15.6	14.26	21.4	0.40	0.39	10.7	6.2	5.61	19.0
121500	87	85.67	1.65	96.2	83.97	16.7	13	17.60	71.3	13	12.1	15.8	10.13	13.44	20.3	0.40	0.37	13.7	4.7	4.66	22.1
121600	84	98.0	5.95	108.5	85.11	13.2	14	10.90	39.7	15	12.3	19.2	12.5	12.76	14.3	0.40	0.35	15.4	5.3	4.59	21.2
121800	86	90.8	11.62	88.5	88.38	13.6	9	10.10	36.3	10	12.6	15.9	11.6	14.30	23.9	0.40	0.39	10.7	4.2	5.36	17.2
121900	87	87.6	7.14	99.5	74.81	15.1	13	10.40	36.3	6	11.3	17.4	13.16	12.32	14.2	0.43	0.36	10.6	6.4	4.50	15.8
122000	85	89.3	7.96	89.7	85.94	14.2	12	11.56	28.7	13	12.11	21.2	12.16	15.89	19.4	0.40	0.42	8.6	5.5	5.06	10.5

Table contd....

Contd. Table 4.38

Somaclone Number	Number of Spikelets/Spike SC Mean	Number of Spikelets/Spike Progeny Mean	Number of Spikelets/Spike Progeny CV %	Peduncle length SC Mean	Peduncle length Progeny Mean	Peduncle length Progeny CV %	Spike length SC Mean	Spike length Progeny Mean	Spike length Progeny CV%	Spike width SC Mean	Spike width Progeny Mean	Spike width Progeny CV %	100 Bur Weight SC Mean	100 Bur Weight Progeny Mean	100 Bur Weight Progeny CV %	Green Fodder Yield SC Mean	Green Fodder Yield Progeny Mean	Green Fodder Yield Progeny CV%	Dry Matter Yield SC Mean	Dry Matter Yield Progeny Mean	Dry Matter Yield Progeny CV%
Explant Seed																					
110100	42	59.50	1.2	23.2	12.85	47.9	7.30	7.70	5.5	1.33	1.01	43.9	0.29	0.44	11.4	40.0	65.0	76.2	15.7	11.75	47.5
110200	99	57.74	38.3	11.5	18.33	22.7	8.03	6.15	14.4	1.20	1.00	9.6	0.40	0.18	21.2	50.0	128.8	60.3	12.0	32.85	65.9
Immature Inflorescence																					
120100	82.3	80.00	-	18.6	12.80	-	7.53	7.13	-	1.16	0.93	-	0.13	0.16	-	80.0	130.0	-	22.4	38.70	-
120300	74.0	53.00	-	17.3	11.50	-	6.56	5.66	-	1.06	0.90	-	0.13	0.14	-	100.0	150.0	-	25.3	38.60	-
120500	54.7	54.08	56.4	21.7	15.30	16.6	5.73	6.61	20.5	0.96	0.89	38.1	0.16	0.19	42.0	120.0	170.0	108.2	30.3	52.00	11.45
120600	36.7	78.66	-	10.4	17.70	-	5.43	6.96	-	0.70	1.10	-	0.23	0.15	-	120.0	150.0	-	29.6	47.20	-
120700	74.0	56.22	16.9	21.5	16.87	27.4	6.46	6.80	11.9	1.10	0.97	7.7	0.17	0.18	13.7	70.0	220.0	32.8	19.1	53.27	21.6
120800	69.7	64.44	34.8	26.5	20.47	17.2	7.30	7.04	22.0	0.90	0.99	20.4	0.13	0.23	32.8	110.0	103.3	106.2	29.7	24.23	126.4
121200	75.66	84.76	27.6	27.1	26.09	9.5	8.33	7.49	11.2	1.13	1.03	8.8	0.11	0.14	34.2	80.0	115.0	41.0	41.7	61.40	33.7
121300	57.50	58.44	29.3	20.5	24.67	12.5	5.15	6.48	19.3	0.70	0.73	16.2	0.16	0.14	72.2	90.0	183.3	41.3	38.0	74.10	35.8
121400	55.33	58.28	21.7	20.6	19.90	14.1	5.50	5.82	18.6	0.83	0.74	17.1	0.12	0.10	31.0	150.0	77.0	41.6	68.7	36.10	40.7
121500	74.00	52.18	36.8	23.9	23.91	13.8	6.30	5.45	15.3	0.83	0.65	19.4	0.12	0.10	23.9	90.0	113.0	61.9	48.9	51.85	47.7
121600	39.66	46.29	37.7	26.9	21.58	21.9	4.56	5.76	19.5	0.63	0.67	17.7	0.07	0.12	30.0	120.0	78.0	36.2	58.0	32.92	37.6
121800	59.66	38.67	35.4	19.1	19.15	21.5	6.20	5.13	30.3	0.73	0.61	19.9	0.12	0.09	41.5	40.0	58.0	63.4	21.9	27.29	60.7
121900	76.33	43.21	33.8	29.5	18.35	24.5	9.20	5.52	18.0	1.03	0.61	16.0	0.15	0.10	19.5	70.0	56.7	38.7	36.3	25.24	38.2
122000	48.00	47.43	42.7	27.2	17.86	37.3	5.43	5.44	22.0	0.63	0.73	20.0	0.09	0.11	42.0	70.0	57.8	55.3	37.8	27.80	56.5

Table 4.39: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, EC400631 during the year 2001.

	Days to flowering			Plant Height			Number of tillers			No. of leaves / tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone Number	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%
Explant Seed																					
110100	56	54.0	-	112.0	121.5	2.9	23.0	35.0	12.1	12.0	12.5	16.9	25.23	23.23	1.0	0.80	0.75	16.1	7.3	7.05	27.1
110200	72	86.53	97.3	103.0	117.08	11.2	18.0	52.7	23.5	16.0	12.1	11.9	25.76	35.90	17.0	0.66	0.61	12.1	6.2	6.28	21.7
Immature Inflorescence																					
120100	70	68.0	-	108.6	123.5	-	41.0	66.0	-	12.0	13.0	-	27.74	40.20	-	0.50	0.60	-	5.5	8.50	-
120300	-	73.0	-	91.0	102.1	-	38.0	42.0	-	10.0	12.0	-	34.83	36.40	-	0.63	0.60	-	8.2	7.30	-
120500	74	72.0	7.2	103.1	105.75	4.4	41.0	61.5	58.6	9.0	11.0	-	32.50	36.07	20.6	0.60	0.60	-	4.5	6.70	19.0
120600	70	86.0	-	108.5	104.20	-	57.0	82.0	-	12.0	14.0	-	31.40	35.20	-	0.53	0.53	-	7.5	6.20	-
120700	79	71.67	1.6	119.1	129.37	4.9	43.0	79.3	31.7	12.0	11.3	13.5	31.06	38.05	8.5	0.56	0.61	2.8	5.5	5.77	11.2
120800	72	63.0	11.1	113.0	98.60	41.7	36.0	30.7	77.2	11.0	11.7	19.8	34.40	32.15	15.3	0.63	0.55	9.2	5.6	4.73	22.8
121200	45	51.22	14.8	140.5	133.54	6.6	46.0	34.1	32.4	13.0	13.2	11.8	43.60	32.55	10.8	0.50	0.57	9.1	8.2	7.67	26.0
121300	-	53.33	28.7	113.6	115.18	6.1	18.0	31.3	25.8	12.0	11.5	11.2	35.86	39.74	9.3	0.56	0.60	13.6	5.3	6.75	45.7
121400	26	45.38	33.1	125.0	126.39	9.4	54.0	30.5	33.0	10.0	14.0	15.1	35.33	34.76	9.4	0.66	0.67	10.3	7.2	6.73	23.6
121500	-	54.33	18.6	126.8	125.50	12.2	19.0	27.2	47.5	13.0	13.4	15.4	34.80	34.38	16.4	0.60	0.57	15.4	7.2	6.93	23.5
121600	-	55.5	24.2	125.8	142.86	9.8	12.0	27.9	27.4	15.0	14.1	13.6	32.73	32.64	8.9	0.50	0.62	12.1	7.4	7.68	21.3
121800	-	49.0	-	118.8	126.43	5.5	19.0	26.9	33.7	11.0	13.2	19.4	30.40	35.76	12.7	0.50	0.63	7.9	7.9	7.07	24.1
121900	36	37.25	15.7	112.7	135.10	9.6	29.0	30.3	43.1	11.0	14.6	21.7	42.90	36.62	13.4	0.70	0.64	8.8	10.2	7.54	16.5
122000	46	40.0	17.0	142.5	131.39	10.3	33.0	30.2	40.3	14.0	13.7	12.7	34.90	37.39	9.6	0.50	0.63	13.1	8.6	7.19	17.6

Table contd.....

Contd. Table 4.39

Somaclone Number	Number of Spikelets/Spike			Peduncle length			Spike length			Spike width			100 Bur Weight			Green Fodder Yield			Dry Matter Yield		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
Explant Seed																					
110100	86.33	120.67	13.3	14.2	17.95	35.1	8.96	10.23	0.4	1.43	1.53	9.2	0.41	0.28	12.4	220	340	26.0	76.50	108.70	12.1
110200	100.30	90.62	28.8	11.6	19.98	28.7	8.90	7.05	19.8	1.00	1.13	13.1	0.21	0.13	25.5	100	429.1	78.7	15.10	119.83	45.4
Immature Inflorescence																					
120100	123.00	74.66	-	17.3	24.56	-	8.70	6.37	-	1.10	1.10	-	0.13	0.11	-	100	260.0	-	25.70	79.80	-
120300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	120	190.0	-	36.25	55.40	-
120500	81.33	82.66	42.8	16.4	19.60	44.0	6.90	7.81	12.9	1.06	1.10	8.4	0.80	0.16	35.4	440	600.0	70.7	128.3	170.90	57.2
120600	119.66	94.33	-	21.5	23.60	-	8.46	7.40	-	1.26	1.06	-	0.09	0.11	-	280	270.0	-	115.0	112.10	-
120700	102.33	79.22	25.8	12.5	23.53	11.7	7.40	6.82	14.0	1.03	1.11	11.6	0.10	0.11	18.4	200	546.7	8.6	63.00	171.23	8.1
120800	92.66	107.00	-	23.4	12.70	-	6.40	6.73	-	1.06	1.06	-	0.09	0.10	-	50	270.0	76.0	12.00	92.10	69.1
121200	89.50	78.15	18.5	14.5	9.98	24.5	8.15	7.38	21.2	0.80	0.83	18.1	0.14	0.21	120.4	580	412.0	59.7	144.64	111.43	46.4
121300	-	61.11	37.4	-	13.73	25.7	-	6.18	28.75	-	0.69	24.6	-	0.14	10.7	130	276.0	50.7	26.05	72.75	35.4
121400	83.66	69.10	18.8	13.1	13.75	24.2	7.73	7.43	9.1	0.83	0.83	16.8	0.13	0.14	26.5	480	253.5	47.3	94.29	70.01	43.1
121500	-	70.27	11.4	-	14.47	11.7	--	6.88	21.1	-	0.72	7.6	-	0.14	11.5	170	210.5	51.0	60.73	64.51	46.3
121600	-	66.41	21.2	-	9.68	33.6	-	6.20	9.7	-	0.82	17.6	-	0.15	29.0	140	241.5	26.0	39.69	72.21	23.8
121800	-	59.33	-	-	10.00	-	-	6.63	-	-	0.60	-	-	0.11	-	120	180.0	75.8	34.73	51.03	62.8
121900	94.5	81.29	9.9	19.1	15.98	22.5	9.0	7.5	12.4	1.10	0.89	14.8	0.18	0.13	16.6	260	222.5	34.6	75.53	61.23	30.7
122000	95.00	77.22	19.1	11.5	17.32	40.2	7.80	7.26	11.9	0.85	0.91	19.4	0.13	0.14	13.0	155	201.0	52.2	39.75	60.98	51.8

length, GFY and DMY. No consistent trend or stability of performance was observed for rest of the traits. Greater variations were recorded for number of tillers per plant, leaf length, number of spikelets per spike, hundred bur weight, GFY, DMY and days to flowering during kharif 2000.

In the year 2001 (table 4.39), the progenies of most of somaclones exhibited better performance with regard to plant height, number of leaves per tiller, leaf length, leaf width hundred bur weight, GFY and DMY but performed comparatively poor for internodal length, spike length and number of spikelets per spike than the respective somaclones. Days to flowering exceeded in majority of the progenies than their parent. Rest of the traits did not show any trend. Greater variation was recorded for number of tillers, peduncle length, hundred bur weight, GFY and DMY.

During year 2000 (table 4.40), the progenies of all somaclones off EC397600 performed better for number of tillers, number of leaves per tiller, leaf length and DMY and comparatively lower with regard to spike width. Days to flowering was found increased in the progenies over the somaclones. Other quantitative traits did not show any trend in the performance. Number of spikelets per spike, GFY and DMY exhibited greater vatriation in this season.

In kharif 2001, progenies of all somaclones consistently recorded better performance for number of tillers and poor performance for leaf length. No trend was observed for rest of the traits. Variation was increased greatly for leaf length and GFY in the progenies.

Table 4.41 indicated a great increase in peduncle lengtrh, spike length, spike width, hundred bur weight and GFY in the progenies of somaclones of the genotype EC400587 during 2000, however, decreased means for plant height, number of tillers, number of leaves per tillers, leaf length, internodal length, DMY and days to flowering were recorded. Greater variations were observed for number of tillers, peduncle length, GFY and DMY during this year whereas during 2001, progenies performed poorly for all the quantitative traits except hundred bur weight. DMY, number of tillers and hundred bur weight were recorded as more variable characters.

Table 4.40: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, EC397600.

KHARIF YEAR 2000

	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
210100	76	79.67	2.9	85.5	88.2	8.8	8.0	9.0	19.3	7.0	7.0	-	23.7	37.75	2.5	0.63	0.84	15.6	17.3	7.87	7.7
210200	94	94.86	3.5	80.6	54.9	27.3	7.0	8.6	20.1	6.0	6.4	15.2	23.0	27.97	16.1	0.73	0.69	10.2	8.6	5.69	41.1
Number of spikelets/spike				Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
210100	74.0	105.3	32.7	19.5	23.7	5.1	7.73	10.22	15.8	1.3	1.23	8.1	0.81	0.74	30.1	55.0	156.67	31.5	13.7	41.47	46.3
210200	86.7	58.0	28.0	22.5	13.5	35.1	8.53	6.68	15.5	1.8	0.87	18.6	0.52	0.58	18.5	90.0	82.86	66.7	15.8	21.51	92.5

KHARIF YEAR 2001

	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
210100	28.0	42.00	6.7	79.5	100.65	3.3	15.0	33.50	2.1	10.0	11.00	12.9	35.83	16.08	22.3	0.50	0.60	-	3.5	4.80	20.6
210200	43.0	38.25	7.0	103.1	91.54	22.5	25.0	37.25	24.8	12.0	10.63	18.1	44.73	41.45	21.7	0.60	0.60	10.0	7.1	3.75	39.1
Number of spikelets/spike				Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
210100	-	11.16	2.1	-	16.9	1.3	-	2.30	-	-	0.93	4.6	-	0.26	10.9	80.0	155.0	31.9	52.4	26.6	6.9
210200	9.66	27.86	113.7	16.5	20.5	33.9	2.0	3.67	73.7	0.9	0.96	16.3	0.16	0.22	78.5	420.0	162.5	36.6	110.3	35.56	13.8

Table 4.41: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, EC400587.

Somaclone number	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
KHARIF YEAR 2000																					
	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
310100	115.0	91.86	3.9	91.5	64.86	16.7	16.0	8.71	26.3	10.0	7.57	19.9	32.8	24.0	18.3	0.70	0.71	13.1	6.20	5.99	20.9
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
310100	65.3	64.16	21.4	5.2	15.84	28.9	6.43	7.64	20.7	0.63	0.98	14.7	0.20	0.54	09.7	90.0	110.0	39.6	26.3	25.44	55.8
KHARIF 2001																					
Somaclone number	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
310100	35.0	34.43	6.63	149.5	100.6	11.7	84.0	38.0	31.2	15.0	11.14	21.6	47.5	40.4	17.8	0.90	0.66	6.8	9.5	4.31	29.1
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
310100	124	60.25	88.8	23.5	22.68	28.8	9.73	6.44	40.9	1.26	0.96	13.7	0.23	0.40	45.5	620.0	248.6	18.9	17.7	63.7	38.5

Increase in the number of leaves per tiller and days to flowering were observed in the progenies of all the somaclones of IG693108 (table 4.42) however, poor performance was observed with regard to plant height, leaf width and GFY. Rest of the characters did not show any trend. Greater variation was recorded for number of tillers, peduncle length, number of spikelets/spike, GFY and DMY during kharif 2000.

In the year 2001 (table 4.43), progenies of all somaclones failed to show any trend for almost all traits except increased number of tillers, internodal length, GFY and DMY. GFY, DMY and number of tillers exhibited greater variation.

The progenies of almost all somaclones of EC400610 (table 4.44) recorded better in performance with regard to number of tillers, number of leaves per tiller, leaf length, peduncle length, hundred bur weight, GFY, DMY. Days to flowering was also recorded to be increased in progenies than the somaclones. No consistent trend in the perfomance were observed for rest of the traits in year 2000. Greater variation for GFY, DMY, hundred bur weight, number of spikelets per spike, peduncle length, internodal length and plant height was observed.

During the year 2001, most of the progenies exceeded the somaclones for number of tillers, number of leaves per tiller, leaf width, spike length, spike width, hundred bur weight, GFY, DMY and days to flowering (table 4.45). No trend was found for rest of the traits. Greater variability for number of tillers per plant. Number of spikelets per spike, GFY, DMY and spike width was recorded.

In the genotype EC397680 (table 4.46), progenies of all somaclones consistently recorded poor performance for height, leaf length, leaf width, internodal length, spike length, number of spikelets per spike, GFY and DMY. Days to flowering increased in the progenies. Specific trends were not observed for rest of the traits during the year 2000. Greater variability was exhibited by number of tillers, peduncle length, spike length, number of spikelets per spike, hundred bur weight, GFY and DMY in this season.

The progenies of all somaclones during the year 2001 (table 4.46) exhibited comparatively lower performance for plant height, humber of leaves, leaf length, internodal length, GFY and DMY than the respective somaclones. No trend was found for rest of the quantitative traits. Number of tillers, GFY, DMY and days to flowering showed greater variability.

(b) *C.setigerus*

A large number of progenies performed better than their somaclones for leaf length and peduncle length whereas comparatively poor performance was recorded with regard to internodal length, spike length, GFY and DMY in the progenies of majority of somaclones of *C.setigerus* EC400639. Days to flowering was also found exceeded in majority of progenies over the somaclones (table 4.47). Rests of the traits did not show any trend of the performance between progenies and somaclones. DMY, GFY, hundred bur weight and number of tillers exhibited great variation during kharif 2000.

The progenies exhibited higher performance with regard to number of tillers, number of leaves per tiller, leaf length, internodal length, peduncle length, GFY, DMY over most of the respective somaclones where as spike length, spike width and number of spikelets per spike were comparatively poorly performed by progenies than their somaclones during the kharif 2001. Variation for peduncle length, internodal length, number of tillers GFY and DMY increased greatly (table 4.48).

(c) *C.echinatus*

The progenies of most of the somaclones of *C.echinatus* EC397342 performed better for plant height, number of leaves per tiller, leaf width, internodal length, peduncle length, spike length, spike width, number of spikelets per spike and hundred bur weight (table 4.49). Days to flowering was reduced in majority of progenies. However, the progenies showed marked reduction in number of tillers per plant. No stability in the performance was observed for the remaining traits. Greater variation was recorded for number of tillers, internodal length, GFY and peduncle length.

Table 4.42: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, IG693108 during kharif 2000.

	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
Somaclone	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Number	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
410100	91.0	93.0	2.1	86.5	79.0	16.8	11.0	9.2	45.2	9.0	9.7	20.1	17.0	15.35	21.5	0.73	0.62	15.9	9.3	6.53	31.9
410200	91.0	92.0	-	62.5	65.5	-	2.0	8.0	-	10.0	9.0	-	2.8	28.83	-	0.30	0.80	-	2.9	3.20	-
410400	84.0	96.0	-	128.0	95.3	-	16.0	11.0	-	9.0	10.0	-	33.5	29.96	-	0.96	0.70	-	9.3	6.50	-
410500	96.0	133.9	25.5	105.0	82.0	16.7	8.0	13.2	21.7	9.0	10.1	15.2	31.1	30.65	19.1	0.70	0.68	9.2	7.1	4.83	17.2
410600	83.0	92.1	9.2	94.6	90.6	17.7	13.0	14.8	37.1	11.0	13.8	29.0	12.0	13.28	17.5	0.50	0.39	15.2	4.2	5.32	16.8
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
410100	53.3	62.77	30.0	21.7	13.4	31.9	4.83	5.49	16.7	1.10	1.02	19.4	0.21	0.22	0.1	90.0	84.0	61.3	17.1	26.55	80.4
410200	32.7	42.00	-	7.8	12.6	-	3.46	6.50	-	0.73	1.10	-	0.19	-	-	8.0	75.0	-	4.6	15.70	-
410400	131.0	50.66	-	18.6	10.6	-	8.83	6.00	-	1.30	0.70	-	0.32	0.15	-	360.0	200.0	-	105.0	45.10	-
410500	62.0	51.40	26.7	13.3	14.8	45.9	6.65	5.75	20.7	0.75	0.91	22.2	0.32	0.21	29.7	180.0	140.0	69.1	44.0	36.96	68.6
410600	69.7	61.66	34.6	20.3	16.2	45.1	9.16	6.36	30.3	1.20	0.84	15.4	0.23	0.14	24.7	90.0	65.5	59.1	43.9	32.33	51.6

Table 4.43: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, IG693108 during kharif 2001.

	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
Somaclone	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
410100	67.0	54.4	10.6	103.0	116.9	6.0	29.0	46.0	20.1	10.0	11.8	15.2	28.6	24.72	17.0	0.5	0.61	5.9	6.6	7.19	18.4
410200	52.0	48.0	-	100.0	34.5	-	78.0	10.0	-	9.0	8.0	-	25.9	23.16	-	0.5	0.36	-	4.5	2.10	-
410400	70.0	71.0	-	155.0	142.5	-	91.0	76.0	-	13.0	15.0	-	40.7	39.46	-	1.0	1.0	-	8.5	7.40	-
410500	-	70.5	12.8	155.0	144.9	8.1	42.0	61.0	47.0	14.0	13.0	10.9	42.1	39.67	7.5	1.0	0.83	14.5	6.5	7.74	17.0
410600	-	32.5	13.4	116.0	124.6	6.0	20.0	27.2	42.5	15.0	13.5	13.6	28.0	35.13	20.1	0.73	0.62	20.1	6.5	7.53	23.3
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
410100	76.7	87.07	16.3	20.5	22.1	21.1	5.46	5.86	73.7	1.13	1.16	7.0	0.22	0.14	21.9	220.0	307.22	25.6	64.8	97.31	26.9
410200	83.3	-	-	18.4	-	-	5.60	-	-	1.06	-	-	0.18	-	-	290.0	8.900	-	62.5	1.60	-
410400	243.7	-	-	19.2	-	-	10.66	-	-	1.36	-	-	0.25	-	-	820.0	650.00	-	208.9	232.60	-
410500	-	156.16	24.8	-	18.3	44.0	-	8.76	40.9	-	1.08	11.7	-	0.12	14.2	420.0	628.33	71.0	128.9	179.12	65.7
410600	-	80.25	5.5	-	20.4	16.4	-	7.44	9.5	-	0.98	5.3	-	0.18	22.7	130.0	210.00	79.3	51.67	76.47	59.6

Table 4.44: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotypes EC400610 during 2000.

Somaclone Number	Days to flowering			Plant hight			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
510300	88.0	97.60	17.5	48.9	56.10	44.9	5.0	13.2	12.0	7.0	7.4	7.4	27.1	22.03	33.0	0.63	0.62	20.0	2.7	4.94	40.4
510500	81.0	96.00	5.6	50.4	44.83	26.1	14.0	15.9	54.1	9.0	6.7	24.4	14.4	20.33	30.9	0.40	0.52	15.3	3.8	3.53	28.3
510600	91.0	97.25	11.3	21.6	63.68	29.9	3.0	7.9	41.1	8.0	8.0	13.3	5.0	16.03	17.8	0.40	0.58	11.9	1.8	5.22	36.0
510700	92.0	92.00	-	35.5	41.50	-	12.0	14.0	-	6.0	6.0	-	11.1	25.36	-	0.40	0.50	-	2.2	4.20	-
510900	96.0	97.83	3.9	23.4	35.32	26.3	10.0	3.8	45.7	4.0	6.2	19.0	13.2	18.06	21.5	0.46	0.50	14.7	2.6	2.98	32.6
511200	93.0	106.67	9.6	37.3	30.19	27.5	7.0	15.6	52.8	6.0	6.1	14.7	14.3	16.83	27.2	0.50	0.45	11.1	3.5	1.74	45.4
511300	94.0	102.38	12.6	60.5	29.55	32.8	26.0	9.4	45.6	6.0	6.0	32.1	28.8	15.91	27.6	0.56	0.50	11.1	4.1	2.66	71.6
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
510300	46.7	59.27	45.2	14.1	16.12	29.6	4.46	5.28	36.4	0.93	0.78	36.0	0.33	0.57	115.6	18.0	94.4	73.3	3.9	22.66	77.9
510500	40.3	54.96	55.3	11.7	14.52	53.9	2.96	4.84	23.8	0.93	0.69	24.3	0.23	0.26	40.0	100.0	56.7	75.6	15.2	11.13	74.8
510600	29.7	42.59	44.7	5.2	16.15	36.9	2.96	5.22	34.0	0.70	0.89	25.4	0.19	0.20	30.8	1.5	31.6	78.2	0.3	9.74	85.2
510700	40.0	33.33	-	11.1	14.70	-	3.56	3.86	-	1.00	0.69	-	0.20	0.36	-	10.0	30.0	-	5.9	7.40	-
510900	19.0	39.67	34.8	10.5	11.07	36.9	2.30	3.58	18.0	0.56	0.69	25.9	0.22	0.32	30.1	4.0	45.8	75.1	1.3	12.86	78.4
511200	29.3	29.14	27.7	14.3	7.14	44.9	3.50	2.61	20.9	0.56	0.52	29.2	0.25	0.23	48.7	15.0	25.6	80.9	4.8	8.07	57.1
511300	67.7	35.08	23.8	22.5	10.02	36.9	5.10	3.09	28.4	0.86	0.67	17.7	0.26	0.25	32.2	120.0	24.4	115.5	27.9	5.47	114.6

Table 4.45: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotypes EC400610 during 2001.

Somaclone Number	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV %
510300	-	48.00	3.0	64.0	86.00	12.8	18.0	52.80	42.3	10.0	10.0	18.7	36.40	36.04	15.4	0.50	0.59	14.8	1.5	4.82	42.0
510500	31.0	42.40	16.2	84.0	82.15	22.0	52.0	49.40	34.6	13.0	11.30	15.1	30.73	34.18	11.4	0.60	0.54	9.6	4.5	3.36	17.1
510600	47.0	52.85	18.8	160.0	159.40	11.3	61.0	64.08	33.7	13.0	15.08	12.8	25.16	25.29	10.2	0.70	0.58	12.1	1.2	7.62	21.9
510700	31.0	53.00	-	51.1	88.50	-	39.0	58.00	-	5.0	9.0	-	27.50	32.16	-	0.66	0.76	-	2.3	4.60	-
510900	47.0	40.83	35.3	63.0	81.73	22.8	28.0	71.67	26.0	8.0	11.50	21.1	20.50	28.38	15.3	0.50	0.56	9.1	2.3	3.65	42.7
511200	34.0	43.57	13.7	78.5	86.57	34.4	43.0	59.43	54.9	7.0	10.57	16.3	29.83	27.06	23.0	0.50	0.55	16.2	4.5	2.94	41.1
511300	44.0	36.63	10.9	110.4	93.25	15.1	146.0	55.00	55.9	13.0	9.00	13.3	32.83	26.81	24.6	0.50	0.52	15.0	4.2	3.81	40.6
	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matte3.81r yield		
510300	-	105.85	31.5	-	25.32	5.5	-	6.85	20.4	-	0.81	20.1	-	0.15	30.0	50.0	216.0	54.8	19.7	69.4	59.1
510500	154.3	95.04	44.9	17.5	25.06	17.3	7.30	6.68	26.5	0.93	0.83	25.1	0.13	0.17	38.6	200.0	188.0	41.2	86.1	52.1	40.4
510600	150.0	94.19	26.0	25.5	19.43	27.8	10.73	8.90	19.3	1.00	1.17	10.5	0.12	0.08	19.4	620.0	413.2	42.0	217.1	147.7	41.0
510700	42.0	88.66	-	7.5	22.10	-	2.85	5.56	-	0.70	0.86	-	0.04	0.04	-	85.0	240.0	-	20.9	60.5	-
510900	51.0	113.94	39.6	21.2	19.38	35.5	3.50	6.55	26.1	0.70	0.82	27.7	0.14	0.24	42.9	100.0	217.7	52.4	24.3	67.0	55.1
511200	57.3	123.60	27.0	26.5	23.12	21.5	4.73	7.47	15.7	0.80	0.92	7.9	0.18	0.29	21.2	90.0	194.8	83.5	20.4	74.1	61.0
511300	152.0	106.50	26.8	20.4	23.40	28.6	7.16	7.42	19.4	0.86	0.89	19.6	0.18	0.27	44.3	500.0	172.5	74.8	153.2	64.4	52.2

Table 4.46: Somaclonal variation for quantitative traits in the progenies of somaclones of the genotype, EC397680.

KHARIF YEAR 2000

	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
610100	84.0	125.0	-	79.8	41.60	-	8.0	13.00	-	11.0	15.00	-	17.8	13.66	-	0.63	0.56	-	6.6	1.50	-
610200	91.0	113.4	12.4	131.2	83.82	13.4	26.0	14.67	41.7	15.0	13.67	10.9	35.7	13.42	18.9	1.26	0.41	5.2	8.3	4.99	19.3
Number of spikelets/spike				Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
610100	90.7	52.00	-	12.1	18.50	-	6.53	6.23	-	0.63	1.10	-	0.28	0.36	-	70.0	40.00	-	18.2	10.00	-
610200	143.3	53.07	38.3	32.1	17.37	32.9	11.90	4.92	35.3	1.03	0.72	23.9	0.22	0.12	30.9	170.0	68.89	23.5	94.0	31.82	28.7

KHARIF YEAR 2001

	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Somaclone number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
	Days to flowering			Plant height			Number of tillers			Number of leaves			Leaf length			Leaf width			Internodal length		
610100	58.0	132.0	-	122.0	99.00	-	82.0	111.0	-	15.0	8.0	-	28.06	27.5	-	0.66	0.70	-	6.5	5.30	-
610200	-	53.7	35.1	159.5	128.3	12.6	70.0	26.8	31.3	15.0	13.6	2.2	59.96	38.71	4.9	1.33	0.81	13.4	10.2	8.35	21.8
Number of Spikelets/spike				Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter yield		
610100	194.6	118.3	-	26.2	30.20	-	9.06	7.70	-	1.4	1.00	-	0.25	0.19	-	895.0	450.0	-	200.8	182.1	-
610200	-	109.5	15.1	-	15.28	18.4	-	7.95	12.1	-	0.94	14.7	-	0.19	15.5	660.0	250.0	33.9	223.2	93.2	33.2

Table 4.47: Somaclonal variation for quantitative traits in the progenies of somaclones of *C.setigerus* 2000.

Somaclone Number	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV %
710100	81.0	78.43	2.8	94.5	100.39	13.0	8.0	14.00	15.4	13.0	12.14	8.8	11.56	12.46	20.6	0.30	0.34	9.3	4.5	5.61	19.1
710400	81.0	79.60	4.0	107.4	92.46	26.1	19.0	22.50	56.9	12.0	11.30	23.6	12.46	13.23	17.5	0.28	0.28	20.7	5.5	5.15	14.0
710500	79.0	80.88	1.4	102.2	111.15	5.6	10.0	17.88	45.1	11.0	11.63	6.4	14.50	15.34	17.1	0.32	0.32	30.3	4.2	5.60	13.4
710600	83.0	83.10	3.4	111.5	96.74	18.0	20.0	12.80	28.9	13.0	12.60	13.6	16.16	13.57	20.1	0.30	0.30	19.7	6.3	4.15	25.9
710700	78.0	80.30	3.6	77.5	94.24	10.7	11.0	17.00	56.9	12.0	12.90	16.9	12.63	18.29	8.4	0.35	0.35	17.5	5.5	5.05	16.3
710800	78.0	81.22	3.2	104.2	91.26	9.4	17.0	12.67	30.3	13.0	12.44	7.1	16.50	15.10	25.3	0.30	0.30	17.6	3.4	4.23	22.1
710900	82.0	80.70	5.5	117.1	103.59	16.7	17.0	15.70	43.7	11.0	11.90	19.9	23.96	19.31	13.5	0.36	0.36	24.9	5.3	4.39	23.3
711000	77.0	80.56	4.2	59.0	96.71	15.9	6.0	12.56	40.8	11.0	12.67	14.2	10.06	17.17	20.9	0.37	0.37	27.9	5.2	4.58	13.1
711200	80.0	82.22	5.2	109.0	120.28	21.8	23.0	18.79	38.9	13.0	14.00	10.7	16.40	17.22	21.8	0.34	0.34	24.1	5.3	4.57	24.9
711300	80.0	83.33	1.9	111.0	95.01	18.1	9.0	13.33	36.2	14.0	11.78	20.7	19.00	17.26	23.6	0.29	0.29	24.0	3.4	4.46	15.6
711400	82.0	84.40	4.4	114.6	84.68	27.2	24.0	12.2	71.1	13.0	11.90	35.8	20.00	18.77	32.9	0.33	0.33	39.8	4.4	4.13	33.1
711500	-	82.90	2.7	40.2	99.02	15.2	3.0	12.90	29.5	7.0	11.60	14.8	13.40	23.08	18.0	0.40	0.40	17.1	2.5	5.10	18.6
711600	78.0	84.13	3.1	110.1	91.36	15.2	13.0	12.63	69.5	11.0	12.00	12.6	14.76	14.89	18.0	0.31	0.31	15.2	5.3	4.82	27.2
711700	83.0	83.10	4.41	70.3	163.29	117.1	16.0	13.90	71.5	11.0	11.20	22.2	10.63	19.59	16.8	0.43	0.43	16.5	2.7	4.94	19.8
711800	82.0	85.38	9.0	113.0	92.51	9.0	23.0	11.56	42.6	11.0	10.75	12.9	21.13	16.04	32.5	0.35	0.35	23.6	5.3	4.65	11.5
711900	83.0	83.50	2.2	90.3	90.61	14.5	13.0	14.20	47.0	7.0	11.30	18.2	13.90	20.31	23.7	0.38	0.38	24..7	4.3	4.08	19.1
712000	87.0	81.88	2.1	10.1	90.85	13.7	5.0	8.50	32.1	12.0	10.00	16.6	15.30	16.08	23.0	0.34	0.34	24.0	2.9	4.47	11.9

Somaclone Number	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV %	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV %
710100	134.00	143.8	0.7	10.3	12.67	34.8	9.16	10.19	6.6	0.73	0.74	26.1	0.70	0.73	29.8	50.0	138.6	20.6	22.2	51.6	21.8
710400	165.66	106.7	26.0	11.6	14.14	19.6	10.60	8.81	17.1	0.70	0.73	9.7	1.02	0.72	30.4	220.0	173.0	97.4	77.5	68.7	96.1
710500	115.33	132.8	15.0	17.4	18.01	13.5	8.90	9.38	5.01	0.86	0.88	7.4	0.64	0.81	20.6	20.0	166.3	36.7	11.3	71.0	54.0
710600	131.33	113.9	12.1	14.5	13.73	20.5	9.6	8.76	9.3	0.73	0.79	13.8	0.95	0.82	22.6	180.0	85.0	46.8	68.5	33.6	43.9
710700	92.00	86.1	15.8	14.6	15.42	17.2	8.70	8.11	9.7	0.73	0.74	19.7	0.74	0.53	31.6	70.0	97.0	67.8	28.9	38.6	59.8
710800	114.66	95.6	21.3	20.3	11.74	22.4	10.00	8.36	10.5	0.93	0.82	13.0	0.77	0.66	33.0	200.0	70.0	42.9	78.8	28.1	41.4
710900	120.00	118.0	42.5	14.0	16.29	32.1	9.40	9.38	13.5	0.86	0.82	15.4	0.52	0.53	23.0	140.0	119.0	66.8	58.9	42.7	68.1
711000	78.66	116.7	25.1	12.1	13.77	15.6	7.13	8.96	12.9	0.73	0.70	14.5	0.42	0.64	42.6	30.0	101.1	52.7	16.5	38.8	49.7
711200	98.66	115.9	26.9	21.8	16.90	16.0	8.93	8.87	14.7	0.80	0.83	13.7	0.60	0.72	14.9	250.0	178.9	63.2	94.5	73.6	52.2
711300	112.66	103.8	22.2	14.1	15.40	20.7	9.46	8.73	12.9	0.83	0.77	10.4	0.53	0.61	31.1	50.0	85.6	43.0	22.3	27.5	52.2
711400	135.00	91.3	24.2	17.2	14.60	27.8	9.33	8.06	21.2	0.83	0.66	20.4	1.02	0.53	27.7	140.0	73.8	85.1	47.0	29.1	70.7
711500	-	111.1	16.9	-	15.39	16.7	-	8.87	9.0	-	0.77	11.9	-	0.49	20.6	3.2	76.0	41.7	1.6	29.2	50.8
711600	148.33	83.5	29.1	12.4	13.43	21.4	9.33	7.08	17.5	0.76	0.60	13.5	0.81	0.52	17.7	150.0	75.8	76.1	54.3	33.8	63.4
711700	92.00	108.2	20.4	10.9	16.40	19.3	7.26	8.37	10.5	0.63	0.69	18.4	0.29	0.63	32.4	50.0	122.0	60.5	23.6	41.7	68.7
711800	82.66	86.7	27.2	13.2	13.68	11.9	8.00	7.60	13.7	0.56	0.64	12.7	0.46	0.42	31.1	200.0	62.5	62.1	79.1	34.2	54.1
711900	77.33	83.0	17.1	12.9	15.12	27.3	8.06	7.61	10.2	0.66	0.61	10.9	0.47	0.48	33.5	130.0	92.2	5.2	52.0	33.6	55.6
712000	66.00	80.0	15.1	13.4	13.87	10.2	7.16	7.25	22.5	0.63	0.54	14.2	0.38	0.38	31.5	90.0	47.3	56.6	38.1	23.3	51.7

Table 4.48: Somaclonal variation for quantitative traits in the progenies of somaclones of *C.setigerus* during year 2001

Somaclone	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
710100	30.0	28.63	13.0	103.4	138.2	13.1	11.0	25.13	33.3	8.0	12.83	20.3	36.46	51.55	12.4	0.66	1.03	17.6	11.5	8.01	21.0
710400	33.0	30.25	14.3	142.5	131.2	9.7	35.0	53.90	77.1	11.0	12.00	14.2	54.73	48.29	15.1	0.93	0.83	11.8	6.7	7.16	25.2
710500	24.0	28.63	8.5	104.0	128.7	13.1	17.0	33.38	31.3	7.0	11.38	21.5	38.86	46.77	13.6	0.50	0.81	14.7	6.9	7.39	15.3
710600	32.0	31.20	15.2	135.5	130.1	21.5	74.0	29.10	43.3	14.0	11.60	17.3	49.50	50.66	19.0	0.73	0.86	18.6	5.7	6.53	22.4
710700	31.0	32.67	11.4	112.3	115.8	7.7	27.0	32.60	48.4	9.0	11.80	18.2	51.56	49.17	6.0	0.80	0.68	10.8	5.5	6.87	22.9
710800	26.0	31.13	18.8	122.4	120.2	9.7	58.0	24.44	55.4	10.0	10.11	21.2	48.33	49.26	12.2	0.83	0.83	15.9	6.6	7.34	12.6
710900	31.0	34.00	19.1	131.6	123.9	6.9	47.0	50.00	47.8	13.0	12.30	15.4	56.06	54.20	8.4	0.76	0.76	10.4	9.7	6.95	17.5
711000	35.0	31.25	3.7	134.5	127.0	9.6	9.0	32.89	45.8	13.0	11.22	18.3	39.38	56.40	11.2	1.13	0.78	12.8	2.3	7.12	18.7
711200	34.0	33.89	19.6	107.5	114.9	10.8	42.0	40.56	56.9	11.0	10.56	27.3	46.53	47.90	19.2	0.66	0.76	21.6	6.6	7.50	26.0
711300	32.0	33.89	7.3	129.3	122.3	8.4	23.0	26.78	43.1	9.0	10.56	19.6	53.16	51.96	15.4	0.70	0.66	16.9	8.5	8.64	57.1
711400	31.0	34.22	7.1	132.4	122.1	10.9	38.0	41.11	42.8	13.0	12.00	16.1	56.06	53.29	5.5	0.70	0.75	15.6	7.2	6.76	32.0
711500	41.0	38.40	26.2	119.4	115.6	8.2	6.0	36.40	32.7	11.0	9.90	20.5	51.06	51.58	8.6	0.60	0.66	14.4	6.6	6.08	31.6
711600	37.0	34.50	14.5	110.5	117.7	17.5	42.0	39.38	58.5	12.0	12.13	18.4	41.70	45.51	7.8	0.60	0.73	23.4	6.3	7.36	59.1
711700	30.0	34.10	13.5	110.6	112.0	10.0	23.0	40.30	51.7	11.0	10.80	17.4	64.60	50.84	8.3	0.66	0.72	11.0	4.5	5.72	31.3
711800	32.0	34.88	35.9	102.9	126.6	16.1	49.0	27.00	23.4	9.0	12.63	13.4	47.23	55.34	8.4	0.70	0.74	14.0	3.6	7.16	25.1
711900	36.0	35.83	5.1	109.6	102.8	8.5	61.0	40.40	45.0	8.0	10.80	19.4	46.16	45.31	11.3	0.63	0.62	14.7	7.4	5.46	33.3
712000	29.0	38.60	19.7	106.4	131.5	11.3	17.0	20.63	13.7	10.0	10.63	18.1	62.70	49.97	7.9	0.70	0.71	16.7	5.2	7.54	49.7

Somaclone	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield			Dry matter		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
710100	30.00	149.3	12.6	5.9	15.11	24.2	6.30	9.75	9.3	0.60	0.74	8.4	0.14	0.54	16.1	80.0	336.3	60.0	28.03	83.47	59.2
710400	145.66	137.9	22.9	7.2	12.11	15.0	9.66	9.19	9.6	0.80	0.65	17.2	0.45	0.45	29.0	440.0	303.0	277.6	96.86	77.12	87.2
710500	97.33	140.0	10.8	17.4	15.59	23.9	8.33	9.16	10.2	0.73	0.69	9.3	0.38	0.43	23.2	80.0	243.8	161.2	20.03	64.27	64.6
710600	138.60	114.9	15.4	12.4	13.16	25.9	8.90	8.34	9.5	0.70	0.65	11.6	0.48	0.37	45.0	300.0	184.0	77.2	81.55	46.53	40.4
710700	121.33	114.0	22.2	13.2	11.61	29.0	8.96	8.76	15.8	0.70	0.69	11.7	0.46	0.51	25.1	100.0	110.0	60.9	26.69	27.20	51.1
710800	127.66	105.6	16.2	8.6	12.53	32.5	9.40	8.55	7.5	0.83	0.66	16.1	0.34	0.48	28.6	260.0	111.1	60.5	73.82	30.95	60.0
710900	105.66	128.6	11.8	12.4	15.71	15.2	8.20	9.46	4.9	0.63	0.70	10.4	0.50	0.51	16.5	280.0	222.5	106.8	153.10	56.26	45.2
711000	80.50	131.7	12.6	9.7	14.63	23.8	6.30	8.95	6.6	0.55	0.66	14.6	0.34	0.50	12.5	50.0	176.1	69.4	12.09	46.98	43.1
711200	173.0	123.2	14.7	14.1	10.22	26.0	10.90	9.03	8.4	0.90	0.63	16.9	0.39	0.52	19.6	140.0	275.6	267.0	39.41	72.89	98.3
711300	139.00	119.1	19.3	19.8	12.97	28.6	8.75	8.34	11.3	0.65	0.66	15.1	0.55	0.52	15.7	80.0	142.2	67.6	22.34	38.72	52.8
711400	138.66	134.1	12.4	20.8	12.32	27.9	10.03	8.95	8.6	0.63	0.66	9.2	0.58	0.50	21.5	150.0	168.9	63.7	40.79	45.02	40.4
711500	171.33	110.1	14.8	20.1	14.72	18.0	10.50	8.61	9.1	0.56	0.67	14.0	0.48	0.47	16.1	30.0	189.5	62.7	9.75	41.86	43.3
711600	155.00	108.6	14.8	9.4	12.18	26.3	9.45	8.42	11.8	0.75	0.62	11.5	0.42	0.45	13.2	180.0	132.5	93.3	45.23	36.09	68.4
711700	140.33	126.5	18.3	13.8	15.79	28.7	9.40	8.88	9.5	0.70	0.69	18.8	0.54	0.46	14.5	100.0	151.0	72.8	27.13	45.22	55.3
711800	146.00	109.4	15.9	15.1	14.29	22.	9.20	8.58	5.7	0.70	0.64	15.3	0.57	0.50	16.0	150.0	166.3	49.3	40.72	47.79	33.4
711900	33.00	106.3	37.6	6.0	1.67	34.2	9.30	8.82	6.42	0.50	0.64	12.1	0.38	0.42	12.9	150.0	126.5	61.5	46.74	36.55	48.7
712000	136.00	103.3	9.2	13.3	11.92	37.7	9.40	8.78	22.4	0.80	0.63	13.3	0.43	0.42	17.1	60.0	82.5	28.7	12.97	23.37	37.4

Table 4.49: Somaclonal variation for the quantitative traits in the progenies of somaclones of *C.echinatus* during year 2000.

Somaclone	Days to flowering			Plant height			Number of tillers			Number of leaves/tiller			Leaf length			Leaf width			Internodal length		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
Number	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
810100	98.0	78.00	21.9	19.5	46.70	5.7	6.0	14.80	42.5	4.0	6.00	11.8	8.26	19.07	21.7	0.66	0.99	13.6	1.1	4.20	35.7
810200	64.0	77.00	4.0	48.5	43.90	20.6	6.0	16.80	36.6	6.0	5.40	10.1	19.23	19.04	9.9	1.00	0.90	12.3	4.7	4.40	41.2
810300	73.0	66.80	7.9	40.6	54.02	4.9	4.0	8.25	26.9	5.0	5.50	10.5	15.13	18.61	14.9	0.83	0.93	9.5	4.1	4.90	23.7
810500	61.0	76.33	3.3	40.2	41.13	10.3	5.0	8.33	81.7	5.0	5.67	10.2	14.23	18.99	11.8	0.86	1.20	21.38	3.1	3.27	38.8
810600	68.0	82.75	3.5	43.2	38.12	15.9	10.0	6.65	39.0	5.0	6.25	24.0	15.36	16.47	31.4	0.80	0.87	7.7	4.6	3.00	39.8
810700	79.0	77.00	18.2	43.0	43.33	13.7	7.0	5.67	79.6	5.0	5.67	20.4	17.20	15.63	35.8	1.13	0.82	22.6	4.3	4.47	32.8
810800	64.0	80.00	9.4	51.5	44.88	22.6	8.0	5.00	46.2	6.0	5.50	10.5	20.20	16.61	15.3	0.80	0.91	4.7	4.2	4.10	21.4
810900	81.0	78.75	5.8	46.0	49.00	11.2	10.0	5.25	39.3	6.0	6.50	19.9	18.03	13.93	8.8	0.86	0.96	18.8	4.5	5.52	21.8
811000	83.0	71.67	7.0	40.0	45.87	26.4	3.0	6.67	22.9	8.0	7.00	14.3	18.26	19.54	11.5	0.83	0.92	7.8	4.5	4.53	12.2
811100	83.0	75.25	10.9	41.6	51.72	18.3	11.0	6.75	53.2	6.0	6.75	14.2	22.00	17.25	17.7	1.00	0.90	6.2	6.4	4.05	25.5
811200	68.0	73.00	17.5	49.0	47.08	9.7	8.0	7.60	57.8	6.0	5.40	10.1	19.16	17.72	20.0	1.00	0.86	5.1	6.3	4.30	11.5
811300	85.0	72.40	16.5	34.1	40.74	13.7	7.0	4.80	22.8	6.0	5.60	9.8	19.90	17.25	11.6	1.10	0.95	10.9	3.5	3.10	44.9
811400	79.0	85.75	3.1	40.2	44.28	19.5	4.0	8.25	23.0	6.0	5.50	18.2	15.06	17.38	9.0	0.86	1.06	7.4	3.2	4.35	23.6
811600	80.0	69.00	6.1	48.5	51.52	25.2	4.0	3.00	62.4	5.0	6.00	11.8	17.23	13.08	22.9	0.83	0.76	9.9	4.9	5.08	16.6
811700	77.0	81.10	7.5	46.4	41.72	16.6	5.0	4.00	46.8	7.0	6.00	20.4	13.66	15.04	27.9	0.86	0.94	12.7	3.2	3.50	21.3
811800	81.0	73.40	10.3	42.5	65.20	8.0	5.0	4.20	39.1	6.0	6.60	8.3	20.03	15.91	9.5	0.70	0.84	13.7	5.6	4.48	9.4
811900	91.0	77.67	9.5	44.5	36.13	33.2	7.0	3.33	45.8	5.0	5.00	20.0	13.00	14.72	15.8	0.86	0.78	8.8	3.4	4.20	27.0
812100	94.0	77.68	5.4	52.5	48.12	12.0	4.0	5.60	39.1	5.0	5.40	10.1	15.73	14.34	5.8	1.00	0.98	5.2	4.2	3.48	11.2
812200	77.0	81.40	4.1	37.7	53.10	14.6	4.0	3.00	62.4	6.0	5.60	16.0	9.00	14.14	19.3	0.76	0.84	7.3	2.9	3.88	18.6
812300	73.0	77.75	6.8	39.5	43.90	12.3	4.0	3.00	54.4	6.0	6.25	20.1	13.80	15.48	21.7	0.60	0.87	24.2	3.1	2.65	25.7
812400	79.0	75.80	6.3	37.5	41.74	39.0	5.0	5.00	58.3	4.0	5.60	20.4	12.93	11.76	18.2	0.90	0.87	11.0	3.2	3.08	51.3
812600	84.0	74.20	6.7	46.5	51.62	10.8	5.0	5.20	25.1	5.0	6.40	8.6	14.10	16.26	16.8	0.90	0.92	17.1	4.5	4.06	38.7
812800	72.0	79.00	5.0	40.2	47.20	15.8	5.0	4.20	45.8	7.0	5.80	7.7	10.50	13.72	15.2	0.76	0.96	10.7	2.7	3.02	17.7
812900	72.0	76.00	3.7	44.5	50.57	13.2	6.0	4.25	11.8	5.0	6.25	8.0	19.40	16.33	27.4	1.13	1.00	5.3	0.5	2.80	36.1
813000	81.0	78.00	6.0	47.5	42.07	37.9	6.0	3.80	43.2	4.0	5.40	10.1	12.43	13.04	17.5	0.90	0.84	13.4	3.1	3.70	36.4

Table contd....

Contd table 4.49

Somaclone Number	Number of spikelets/spike			Peduncle length			Spike length			Spike width			100 Bur weight			Green fodder yield		
	SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny		SC	Progeny	
	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%	Mean	Mean	CV%
810100	16.66	29.33	8.4	3.3	16.30	34.8	4.26	7.01	8.6	0.86	1.33	10.2	1.10	2.32	9.0	10.0	14.20	26.5
810200	31.00	36.50	25.8	19.2	13.42	34.8	5.70	7.13	11.8	1.10	1.37	14.6	2.20	2.22	16.7	60.0	132.0	54.5
810300	21.00	26.00	7.3	-	21.15	18.1	5.60	6.42	6.1	1.10	1.29	15.0	2.03	2.30	15.1	20.0	142.5	35.0
810500	14.00	17.33	14.5	12.7	14.78	27.1	5.30	5.61	17.2	1.20	1.12	6.8	1.60	2.03	12.4	60.0	100.0	87.2
810600	12.33	17.21	19.0	24.4	11.63	25.4	4.80	6.13	21.8	1.15	1.19	1.1	2.00	1.88	24.4	90.0	77.5	37.1
810700	22.00	18.22	29.8	23.0	18.73	25.7	6.10	5.75	17.4	1.00	1.20	11.2	2.70	3.27	14.5	110.0	73.3	67.3
810800	25.00	23.71	21.5	16.5	15.73	33.2	6.33	6.34	13.4	1.16	1.28	14.2	3.00	2.80	19.1	120.0	85.0	20.4
810900	22.00	22.91	12.1	12.0	16.38	11.0	6.20	6.10	12.7	1.16	1.07	5.2	2.60	2.75	11.3	170.0	110.0	42.6
811000	12.00	22.89	11.9	13.1	18.50	16.4	4.50	6.14	7.7	1.00	1.13	5.1	2.20	2.27	25.1	30.0	-	-
811100	22.00	22.16	6.3	15.0	21.50	22.9	6.83	6.07	4.2	1.20	1.15	3.7	2.60	3.07	18.1	200.0	137.5	41.7
811200	22.66	18.20	30.8	16.2	16.80	27.6	5.93	5.55	15.7	1.20	1.06	11.6	2.80	2.62	18.6	200.0	105.0	63.2
811300	9.00	16.10	25.1	8.3	14.86	19.8	3.66	5.36	23.8	1.00	1.08	8.1	2.80	2.26	22.3	100.0	95.0	10.8
811400	14.66	22.79	6.9	13.1	13.90	20.8	4.70	5.92	16.8	0.90	1.27	13.1	1.90	2.55	12.2	40.0	105.0	35.2
811600	12.50	18.00	26.1	12.5	18.28	53.3	6.70	5.70	21.1	0.83	1.17	9.7	1.70	2.68	25.2	80.0	74.0	68.0
811700	19.33	21.53	8.2	12.5	16.20	19.4	5.13	6.13	11.4	1.16	1.23	7.5	2.50	2.02	25.8	50.0	108.0	86.0
811800	27.00	26.40	6.6	6.3	28.39	13.8	6.70	7.22	3.6	1.30	1.32	7.8	1.80	3.20	24.9	40.0	162.0	42.2
811900	18.66	18.50	11.8	13.3	7.63	67.2	5.90	5.79	18.8	1.36	1.18	12.2	3.40	2.53	16.4	100.0	76.7	96.1
812100	22.00	25.46	8.1	15.5	20.48	20.1	5.80	6.43	10.1	1.30	1.25	13.0	2.00	1.98	34.9	50.0	80.0	54.5
812200	16.00	22.16	10.0	12.4	22.62	8.0	5.36	6.32	5.7	1.30	1.28	4.6	1.70	2.34	22.5	20.0	71.0	64.8
812300	22.00	19.38	25.4	10.2	16.10	15.4	5.75	4.74	26.4	1.20	0.94	10.4	1.02	2.45	10.3	40.0	53.0	62.4
812400	15.33	21.13	36.1	21.3	16.22	46.7	4.36	5.94	20.9	1.16	1.16	15.3	2.90	2.32	11.2	50.0	124.0	53.7
812600	19.00	21.43	15.9	12.5	18.84	23.9	6.70	5.98	11.2	1.50	1.31	5.2	1.50	2.82	14.7	60.0	114.0	42.8
812800	14.00	18.53	3.5	11.4	13.68	24.6	5.60	5.67	10.2	1.15	1.25	6.1	1.50	2.10	42.3	70.0	62.4	95.6
812900	23.00	25.74	20.4	14.2	19.17	17.7	5.30	6.63	20.4	1.60	1.20	9.7	1.80	2.13	19.0	145.0	76.3	47.7
813000	18.00	23.23	25.4	16.5	17.08	41.4	5.50	6.13	35.2	1.20	1.16	12.9	2.00	2.46	47.0	150.0	50.0	83.7

4.2.1.1 Qualitative Variations

The somaclonal variations are of greater utility if desirable changes in their morphological or qualitative traits or increments in yield and its component are brought about. The first category of these variations, *viz.*, morphological/phenotypic/qualitative variations were studied. The variations pertain to growth habit, leaf surface and bur colour. Such variations are presented progenty wise in table 4.50 to 4.52.

(a) **Growth habit**

Table 4.50 indicated the nature of growth habit shown by plants, somaclones and their progenies of *C.setigerus, C.echinatus* and different genotypes of *C.ciliaris*. Most of the parents exhibited semi-erect habit during the season of 2000 and 2001. Most of the parents of genotypes EC400631 (78.5%) indicated semi-erect habit and its 89.47 per cent somaclones and their 90.90 per cent progenies were also semi-erect during the year 2000. However, in year 2001, the percentage of semi-erect habit was some what reduced in somaclones and their progenies. In the genotype IG693108 also most of the plants, somaclones and their progenies were found to be semi-erect except 2.94 per cent of progenies with prostrate and 2.94 per cent with erect nature in the year 2000, where as during year 2001, increased proportion of prostrate plants (16.6%) were recorded. Majority of the plants of the genotypes, EC400610 and EC397680 including parent, somaclones and their progenies showed semi-erect growth habit, however, some plants of prostrate nature were also observed. Seasonal effect was not prominent on the genotype EC397680.

In case of *C.setigerus* also the parent, somaclones and progenies were found totally to be semi-erect in both the seasons (table 4.50). In case of *C.echinatus* most of the parent plants were prostrate in nature (80%). Its 41.66 per cent somaclones exhibited prostrate nature where as their progenies were also found closer to prostrate in nature (64.39%). None of the somaclones of any genotype was found to be erect, however, some progenies of somaclones of EC400631, IG693108 and EC400610 showed erect, habit of growth.

(b) Leaf surface

The percentage of plants with smooth surface leaves was greater (57.14%) in the genotype. The results exhibited by its somaclones were vice versa during the year 2000 and 2001 (table 45), however their progenies showed similar trend to parent (EC400631). 50 per cent somaclones and their 66.66 per cent progenies of the genotype EC397600 had leaves with rough surface while the parent had 75 per cent rough leaves. The percentage of plants with rough leaves (81.81%) was higher in the genotye IG693108 while all of its somaclones had smooth leaves and their 58.82 per cent progenies showed smooth leaves. Somaclones and their progenies of the genotype EC400610 indicate similar trend. In the genotype EC397680 majority of plants (75%) had smooth leaves while its somaclones showed rough leaf surface but their 80 per cent of progenies contained smooth leaves.

While, *C.setigerus* and their somaclones showed similar trend of having rough leaves, almost 50 per cent of the progenies with smooth leaves were observed. In *C.echinatus*, 80 per cent parent had smooth leaves while 56.6 per cent somaclones and 55.3 per cent of their progenies of *C.echinatus* had smooth leaves.

(c) Bur colour

Somaclones and their progenies of the genotypes EC400631, IG693108 and EC397680 of *C.ciliaris* exhibited green bur colour during the year 2000 and 2001 (table 4.52) while genotype EC397600 of *C.ciliaris, C.setigerus* and *C.echinatus* contained purplish pink color of bur. The genotype EC400587 and its 50% somaclones exhibited green burs whereas the remaining 50% somaclones and their 70 per cent progenies showed purpilish pink burs (plate # 17, fig. 2). On the other hand the genotype EC400610 and its somaclones had purplish pink burs whereas their 5.6 per cint of progenies had green burs.

4.2.1.3 Inheritance of somaclonal variation

Per cent deviation from parent to somaclones and their progenies, a measure of incidence of variation due to tissue culture revealed effect of *in vitro* culture in

Plate # 17

fig-1 EC400631 *(C. ciliaris)*

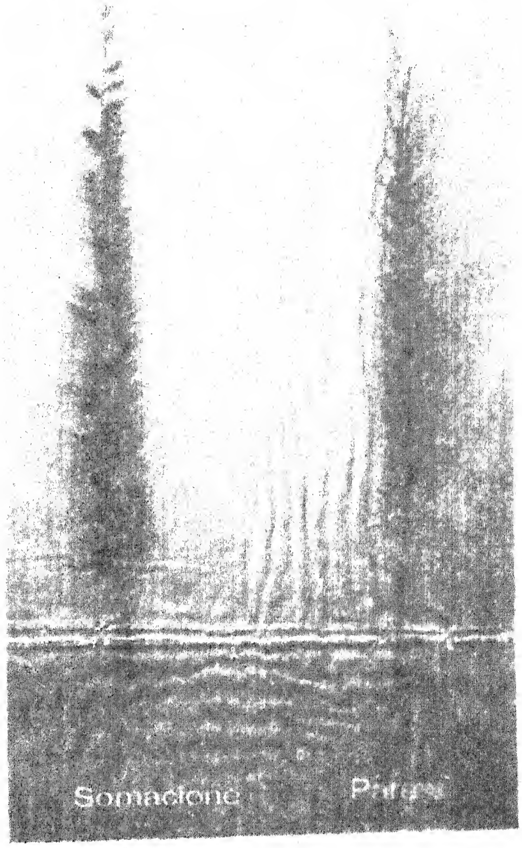


fig-2 Mature inflorescence
EC400587 *(C. ciliaris)*

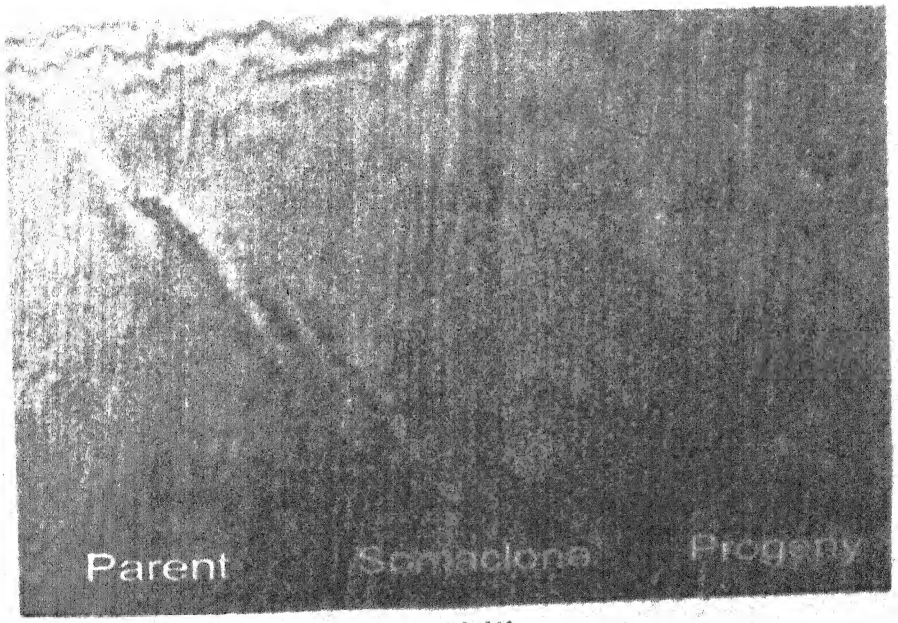


fig-3 Variation in leaf width
EC397680 *(C. ciliaris)*

Table 4.50: Frequency distribution of plants in parent, somaclones and their progenies for growth habit.

Genotype	Growth habit	Kharif 2000						Kharif 2001					
		Parent		Somaclone		Progeny		Parent		Somaclone		Progeny	
		# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)
C. ciliaris													
EC400631	prostrate	2	14.28	2	1052	6	5.45	2	14.26	2	13.3	20	18.18
	semierect	11	78.57	17	89.47	100	90.90	11	78.57	17	86.66	68	61.81
	erect	1	7.14	0	0.00	4	3.63	1	7.14	0	0.00	22	20.0
EC397600	prostrate	1	25	1	50	2	16.66	1	25	1	50	2	16.66
	semierect	3	75	1	50	7	58.33	3	75	1	50	7	58.33
	erect	0	0.00	0	0.00	3	25.0	0	0.00	0	0.00	3	25.0
EC400587	prostrate	0	0.00	0	0.00	6	60	0	0.00	0	0.00	6	60
	semierect	1	100	1	100	3	30	1	100	1	100	3	30
	erect	0	0.00	0	0.00	1	10	0	0.00	0	0.00	1	10
IG693108	prostrate	0	0.00	0	0.00	1	2.94	0	0.00	0	0.00	1	2.94
	semierect	11	100	5	100	32	94.11	11	100	5	100	32	94.11
	erect	0	0.00	0	0.00	1	2.94	0	0.00	0	0.00	1	2.94
EC400610	prostrate	1	14.28	1	16.66	21.12	21.25	1	14.28	1	16.66	15	21.12
	semierect	6	85.7	5	83.33	67.60	78.61	6	85.7	5	83.33	48	67.60
	erect	0	0.00	0	0.00	11.26	13.11	0	0.00	0	0.00	8	11.26
EC397680	prostrate	1	25	0	0.00	1	10	1	25	0	0.00	1	10
	semierect	3	75	2	100	9	90	3	75	2	100	9	90
	erect	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
C. setigerus	prostrate	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	semierect	10	100	20	100	157	100	10	100	20	100	156	100
	erect	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
C. echinatus	prostrate	4	80	10	41.66	85	64.39						
	semierect	1	20	15	58.33	42	31.81						
	erect	0	0.00	0	0.00	5	0.03						

Table 4.51: Frequency distribution of plants in parent, somaclones and their progenies for leaf surface

Genotype	Leaf surface	2000 Parent		2000 Somaclone		2000 Progeny		2001 Parent		2001 Somaclone		2001 Progeny	
		# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)
C. cilliaris													
EC400631	Rough	16	42.85	17	89.47	40	36.36	6	42.85	17	89.47	40	36.36
	Smooth	8	57.14	2	10.52	70	63.63	8	57.14	2	10.52	70	63
EC397600	Rough	3	75	1	50	8	66.66	3	75	1	50	6	50
	Smooth	1	25	1	50	4	33.33	1	25	1	50	6	50
EC400587	Rough	1	100	1	100	10	100	1	100	1	100	10	100
	Smooth	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
IG693108	Rough	9	81.81	0	0	14	41.17	9	81.81	1	20	14	41.17
	Smooth	2	18.18	5	100	20	58.82	2	18.18	4	80	20	58.82
EC400610	Rough	1	14.28	1	16.66	19	26.76	1	14.28	1	16.66	19	26.76
	Smooth	6	85.74	5	83.33	52	73.23	6	85.79	5	83.33	52	73.23
EC397680	Rough	1	25	2	100	2	20	0	25	2	100	2	20
	Smooth	3	75	0	0.00	8	80	8	75	0	0.00	8	80
C. setigerus	Rough	6	60	13	65	79	50.31	6	60	13	65	82	52.56
	Smooth	4	40	7	35	78	49.68	4	40	7	35	74	47.43
C. echinatus	Rough	1	20	11	44	59	44.69						
	Smooth	4	80	14	56	73	55.3						

Table 4.52: Frequency of plants, somaclones and their progenies for bur colour

		2000						2001					
Genotype	bur colour	Parent		Somaclone		Progeny		Parent		Somaclone		Progeny	
		# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)	# Plants	Percentage (%)
C. ciliaris													
EC400631	Green	14	100	19	100	110	100	14	100	19	100	110	100
	Purple	0	0	0	0	0	0	0	0	0	0	0	0
EC397600	Green	0	0	0	0	0	0	0	0	0	0	0	0
	Purple	4	100	2	100	12	100	4	100	2	100	12	100
EC400587	Green	1	100	1	100	3	30	1	100	1	100	3	30
	Purple	0	0	0	0	7	70	0	0	0	0	7	70
IG693108	Green	11	100	5	100	4	100	11	100	5	100	34	100
	Purple	0	0	0	0	0	0	0	0	0	0	0	0
EC400610	Green	0	0	0	0	4	5.6	0	0	0	0	4	5.6
	Purple	7	100	6	100	67	94.36	7	100	6	100	67	94.36
EC397680	Green	4	100	2	100	10	100	4	100	2	100	10	100
	Purple	0	0	0	0	0	0	0	0	0	0	0	0
C. setigerus	Green	0	0	0	0	0	0	0	0	0	0	0	0
	Purple	10	100	20	100	156	100	10	100	20	100	156	100
C. echinatus	Green	0	0	0	0	0	0	0	0				
	Purple	5	100	25	100	149	100	5	100				
	erect	0	0.00	0	0.00	5	0.03						

three genotypes of *C.ciliaris* and one each of *C.setigerus* and *C.echinatus* (Table 4.53).

Highest deviation was found in somaclones of *C.setigerus* for days to flowering (100%) followed by spike length, hundred bur weight and spike width (95%). Deviation ranged between 0 per cent in *C.echinatus* for plant height, peduncle length, spike length and GFY to 100 per cent in somaclones of *C.setigerus* for days to flowering. Leaf width and days to flowering (88%) were measured as most deviated characters in *C.echinatus* followed by the peduncle length.

In the somaclones of *C.ciliaris* maximum deviation was recorded for number of tillers and GFY (90%) in genotype EC400631 while minimum deviation was observed for the trait, hundred bur weight (10%) in the same genotype. Somaclones of IG693108 were highly deviating for the trait, hundred bur weight (75%). The maximum deviation for internodal length (75%), plant height, number of leaves per tiller, peduncle length and spike width (62%) and the minimum deviation for number of tillers and hundred bur weight (12.5%) were recorded in somaclones of the genotype EC400610. Less than 50 per cent deviation was recorded for rest of the traits in somaclones of this genotype.

In the progenies of *C.ciliaris,* deviation was varied between 10.31% for hundred bur weight to 92.31% for number of tillers among all the genotypes. In the genotypes EC400631 highly deviating characters were number of tillers (92.31%). GFY (88.46%) and DMY (85.58%). The minimum deviation was recorded for hundred bur weight (10.31%). In the genotype IG693108, maximum deviation was recorded for days to flowering (86.21%) and minimum for peduncle length (13.79%). Genotype EC400610 exhibited higher deviation for the trait, number of leaves per tiller (75.76%) followed by plant height (68.18%), peduncle length (66.67%), leaf length (65.15%) and internodal length (62.16). The minimum deviation in this genotype was recorded for number of spikelets per spike (13.64%). In case of *C.setigerus* maximum deviation was recorded for hundred bur weight (96.75%) and the minimum deviation was recorded for leaf length (51.59%). The deviation for rest of traits among the progenies ranged from 93.5% to 69.43%. In case of *C.echinatus*, deviation ranged between 95.45% for leaf width and 0% for plant height, number of spikelet per spike, spike length and GFY in the

Table 4.53: Percentage deviation from parent to somaclones and their progenies over two seasons in *Cenchrus*

Characters	*C. ciliaris*						*C. setigerus*		*C. echinatus*	
	EC400631		IG693108		EC400610		EC400639		EC342347	
	Somaclone	Progeny	Somaclone	Progeny	Somaclone	Progeny	Somaclone	Progeny	Somaclone	Progeny
Days to flowering	70	58.65	60	86.21	25	44.93	100	92.36	88	94.74
Plant height	50	57.69	40	18.75	62.5	68.18	70	69.43	0	0
Number of tillers	90	92.31	40	71.88	12.5	34.85	70	71.34	20	21.21
Number of leaves/tiller	30	36.5	20	37.5	62.5	75.76	80	77.07	52	62.12
Leaf length	75	79.81	4	25	25	65.15	25	51.59	60	61.36
Leaf width	25	15.38	60	46.88	60	46.88	75	88.54	92	95.45
Internodal length	60	60.58	60	31.25	75	62.16	90	88.54	60	65.91
Number of spikelets/spike	70	55.67	20	55.17	25	13.64	90	90.91	0	0
Peduncle length	65	66.59	20	13.79	62.5	66.67	80	84.62	80	22.73
Spike length	70	57.73	40	41.38	37.5	45.45	95	92.21	0	0
Spike width	70	59.79	60	72.41	62.5	52.94	95	93.51	28	56.06
100 Bur weight	10	1031	75	60.71	12.5	24.24	95	96.75	12	23.48
Green fodder yield	90	88.46	60	65.63	25	31.82	65	77.07	0	0
Dry matter yield	75	85.58	40	53.13	37.5	27.27	65	75.8	20	57.12

progenies. Days to flowering (94.74%) also noted as highly deviating character. The deviation for rest of the characters ranged from 65.9% to 21.21%.

Frequency distribution of somaclones and their progenies over two seasons

The frequency distribution of somaclones and their progenies over two seasons with respect to the variations exhibited in terms of quantitative Characters is presented in fig. 2 to fig. 15 for all the three *Cenchrus* species studied. It is quite evident from these figures that the type of distribution and the mode differed between somaclones, progenies and genotypes in several cases. However, in some cases, progenies exhibited coagulation around the distribution and mode of the somaclones hence indicated the stabilization of variation.

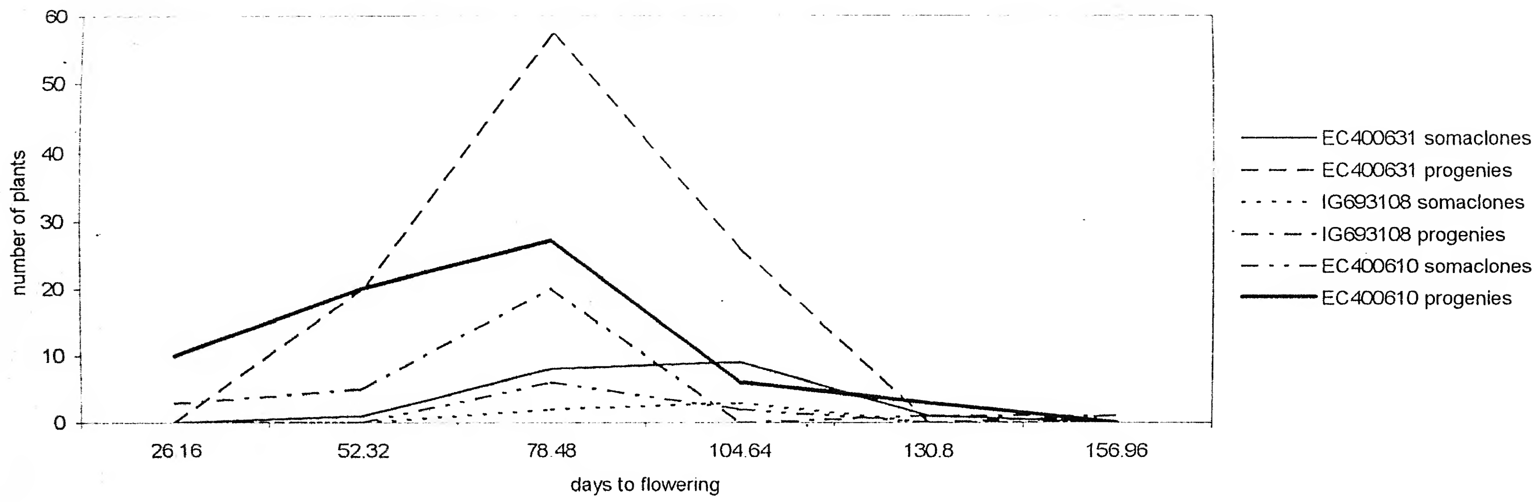
- **Days to flowering**

  Among the *C.ciliaris* genotype (fig.2) frequency distribution of variants and their mode for days to flowering was entirely different in somaclones and progenies of EC400631 where mode of distribution of somaclones was skewed and progenies clustered around the mode indicating stabilization of variation. With unimodal and almost symmetric distribution, progenies were highly contrasting in mode of distribution from that of somaclones of IG693108. However, in case of EC400610, type of distribution was similar in somaclones and progenies.

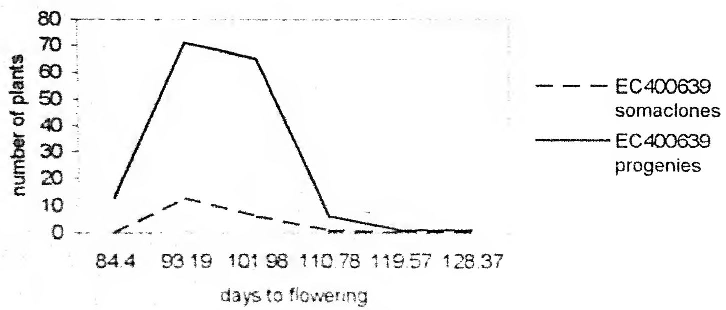
  C.setigerus genotype, EC400639 exhibited different mode of distribution for days to flowering in somaclones and their progenies. The pattern of frequency distribution was skewed in progenies compared to that of somaclones. Similarly for *C.echinatus* genotype, EC397342, the distribution of variation for days to flowering and its mode were different in somaclones and the progenies. Progenies exhibited almost equal distribution in two adjacent classes (fig.2).

- **Plant height**

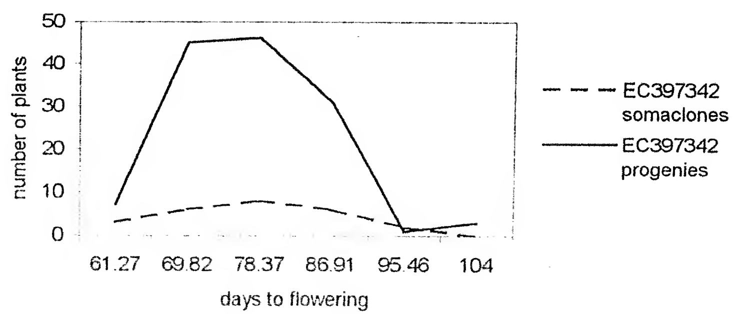
  For plant height, the genotype, EC400631 showed similar distribution of variation and its mode somaclones and progenies (fig.3). EC400610 also recorded similar distribution for plant height and its mode in somaclones and



C.ciliaris

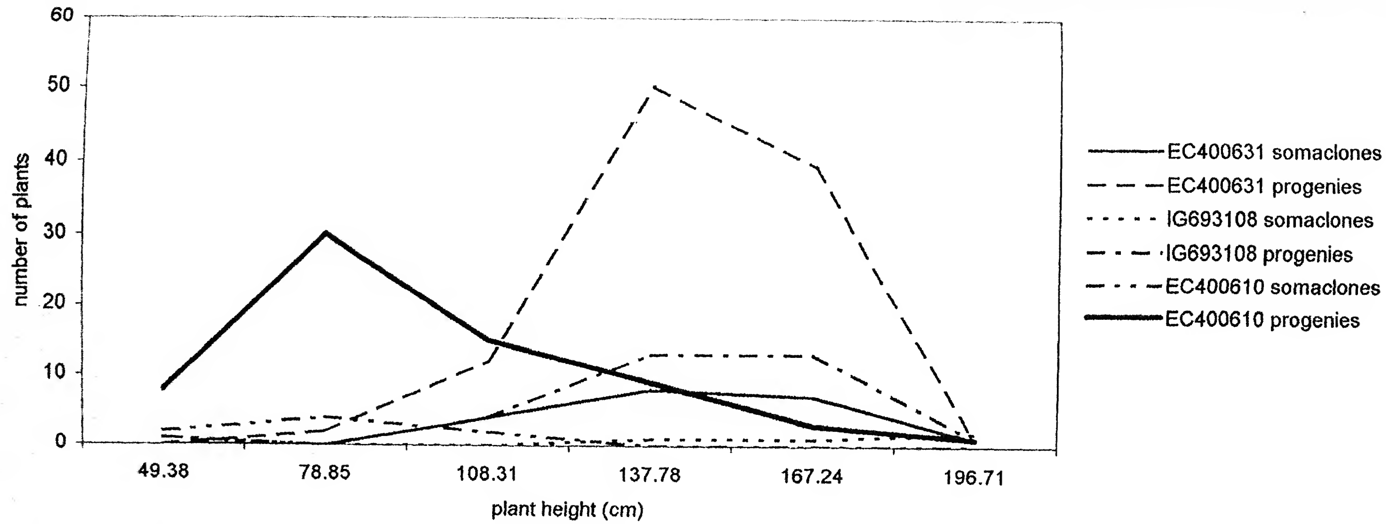


C.setigerus

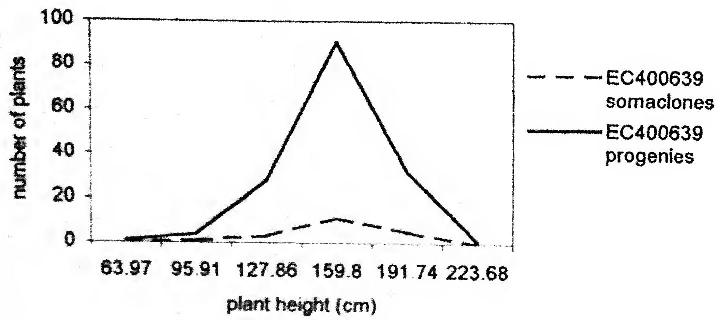


C.echinatus

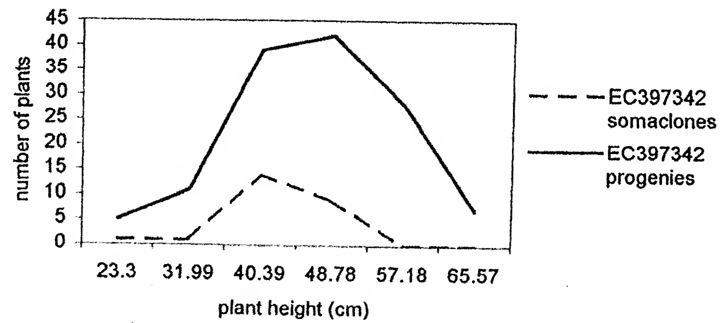
Fig 2 Frequency distribution of somaclonal variation for days to flowering in somaclones and their progenies



C.ciliaris

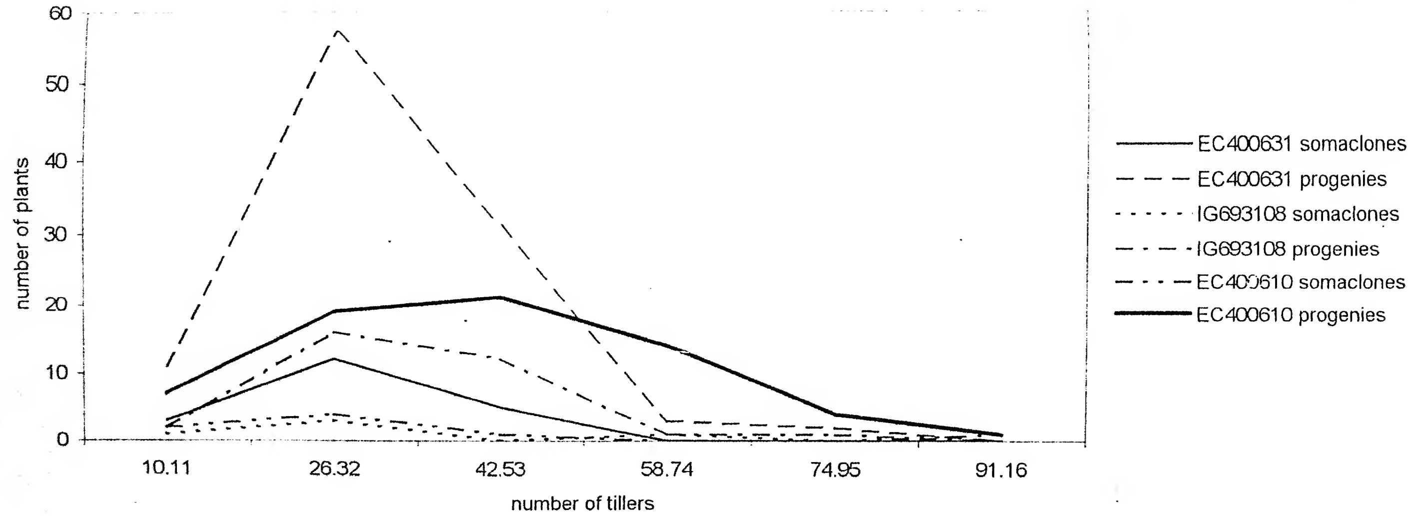


C setigerus

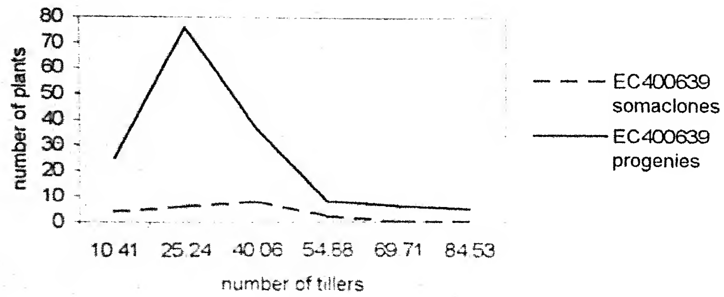


C.echinatus

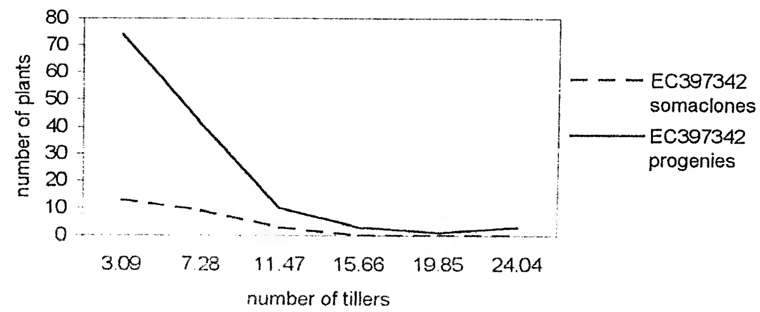
Fig 3 . Frequency distribution of somaclonal variation for plant height in somaclones and their progenies



C. ciliaris



C setigerus



C. echinatus

Fig 4 : Frequency distribution of somaclonal variation for number of tillers in somaclones and their progenies

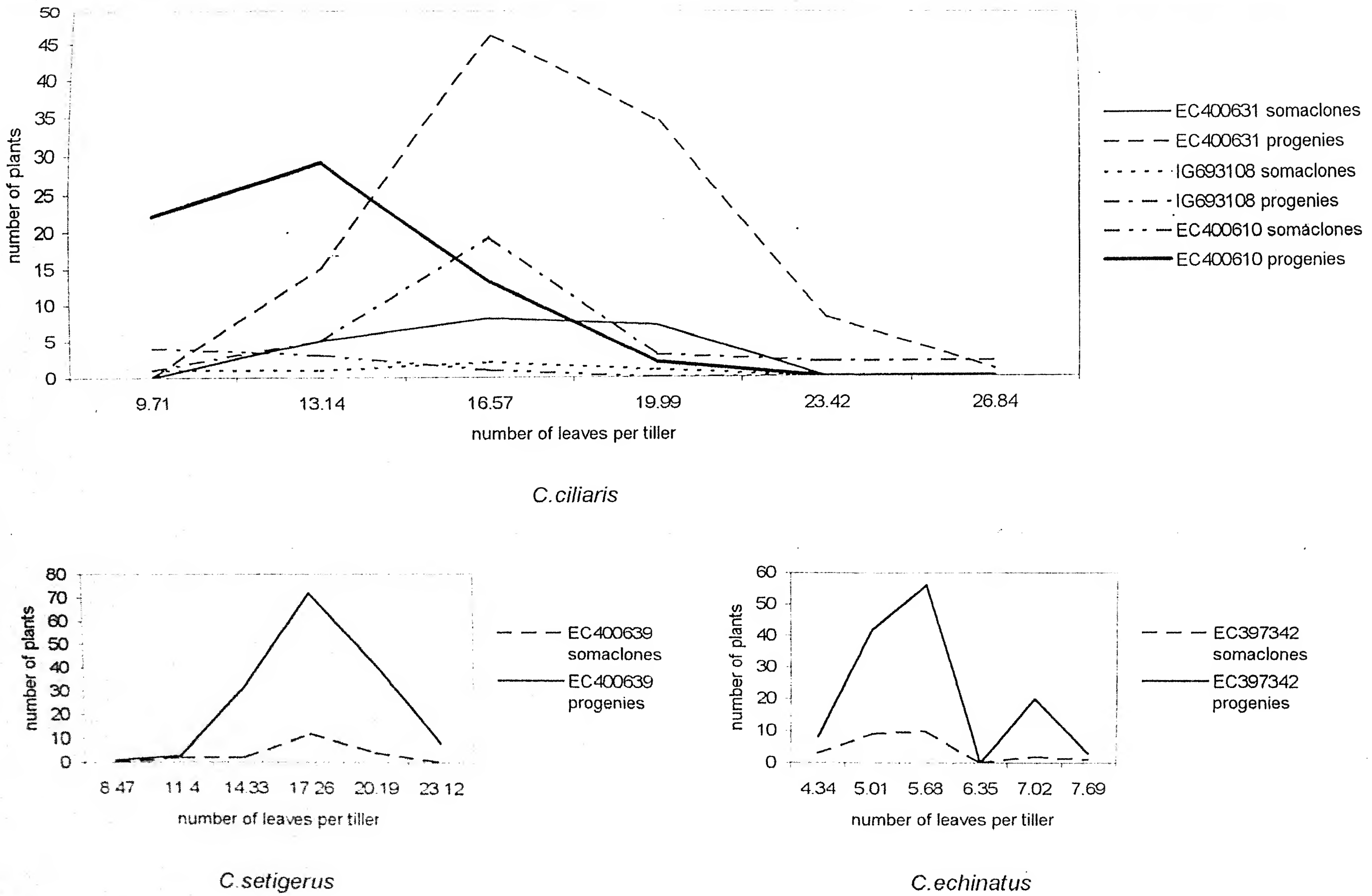


Fig 5 : Frequency distribution of somaclonal variation for number of leaves per tiller in somaclones and their progenies

progenies, however, falling of progenies was more in the class with lesser plant height. Different mode of distribution was observed with a symmetric and skewed distribution of progenies in the *C.ciliaris* genotype IG693108.

Type of distribution was similar in *C.setigerus* genotype EC400639 in somaclones and progenies coagulated around the mode indicating the stabilization of variation. Entirely different type of distribution was noticed in *C.echinatus* genotype EC397342. Somaclones exhibited unimodal distribution with less frequency of variants at higher plant height while mode of distribution in progenies was skewed (fig.3)

- **Number of tillers**

Among *C.ciliaris* genotypes, EC400610 recorded very similar distribution of variation for number of tillers and its mode in somaclones and progenies (fig.40. In case of EC400631, the type of distribution was quite similar in somaclones and progenies and most of the progenies tended to stabilize around the mode of distribution. However, different type of distribution with downward shift in the mode of progenies was observed for the number of tillers of *C.ciliaris* genotype IG693108.

Progenies crowded around the mode indicating stabilizing of variation and exhibited less frequency of variants at the upward side of the distribution of number of tillers compared to that of somaclones of C.setigerus genotype EC400639. The mode of progeny distribution was similar to that of somaclones for number of tillers in the *C.echinatus* genotype EC397342 (fig.4).

- **Number of leaves per tiller**

Though the type of distribution was asymmetrical for number of leaves per tiller (fig.5) in somaclones and progenies of EC400631, the similar pattern of distribution was recorded for the both. In case of IG693108, progenies to be inclined to stabilize around the mode of distribution with unimodal and symmetric distribution for number of leaves per tiller compared to that of somaclones. Gradual decrease in frequency of variants was noticed with higher frequency class in progenies as well as in somaclones of EC400610 of *C.ciliaris*.

Progenies clustered around the mode to indicate the stabilization of variation and represented less frequency of variants at the downward side of the distribution of number of leaves per tiller compared to that of somaclones in *C.setigerus* genotype EC400639. Entirely contrasting trend was observed in *C.echinatus* genotype EC397342 where progenies exhibited bimodal distribution of variants (fig.5)

- **Leaf length**

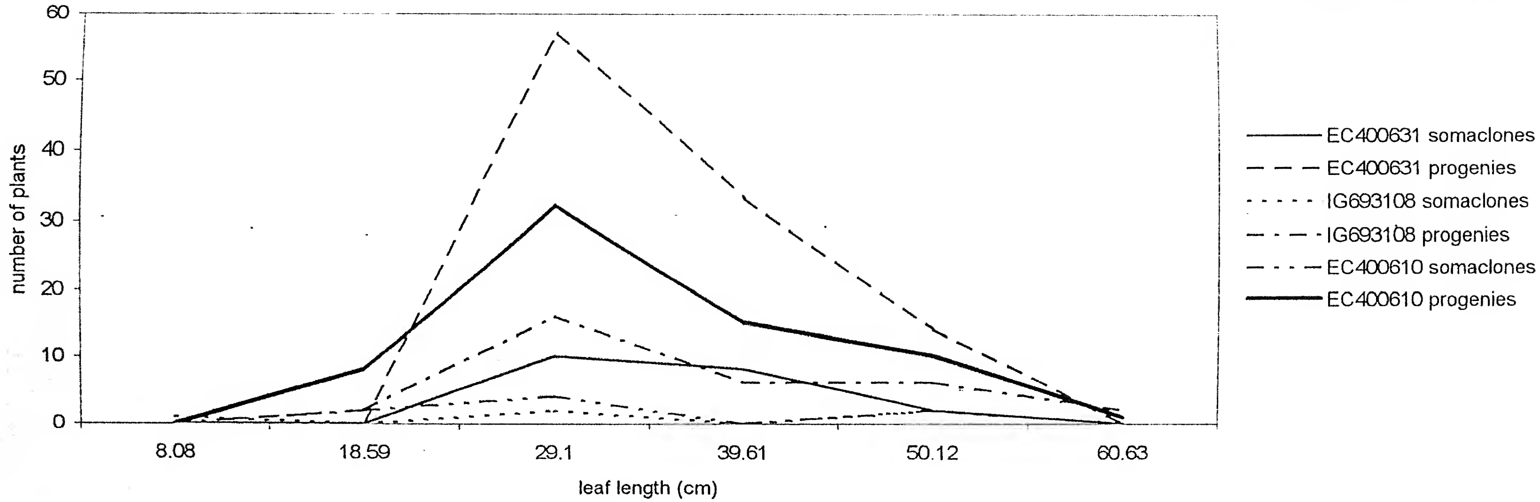
The recording of leaf length in *C.ciliaris* genotype EC400631 somaclones displayed asymmetric and different mode of distribution compared to their progenies. Progenies exhibited unimodal distribution with coagulation around the mode to indicate the stabilization of variation. IG693108 recorded very similar distribution of variation for leaf length and its mode in both somaclones and progenies. However, most different type of distribution with upward shift in the mode of progenies was noticed for leaf length in *C.ciliaris* genotype EC400610 (fig.6).

Most contrasting trend was found in *C.setigerus* genotype EC400639, where more progenies being represented at higher leaf length with unimodal distribution. In case of *C.echinatus* (EC397342), somaclones and progenies were different in mode of distribution with bimodal distribution of somaclones (fig.6).

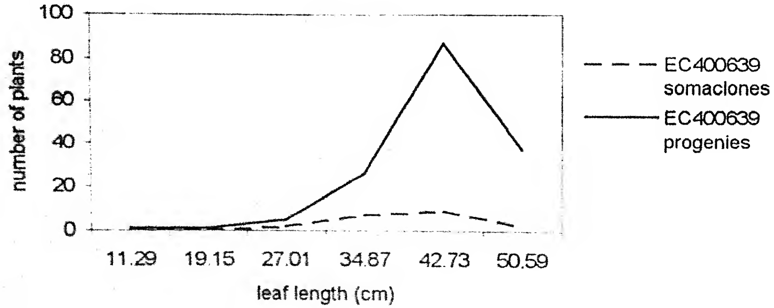
- **Leaf width**

Among the *C.ciliaris* genotypes, EC400610 recorded very similar distribution of variation for leaf width and its mode in both somaclones and progenies. In case of EC400631, the type of distribution was similar in somaclones and progenies and the progenies crowded around the mode indicating stabilization of variation. However, entirely different type of distribution with an upward shift in the mode of progenies was recorded for the leaf width of *C.ciliaris* genotype IG693108 (fig.7).

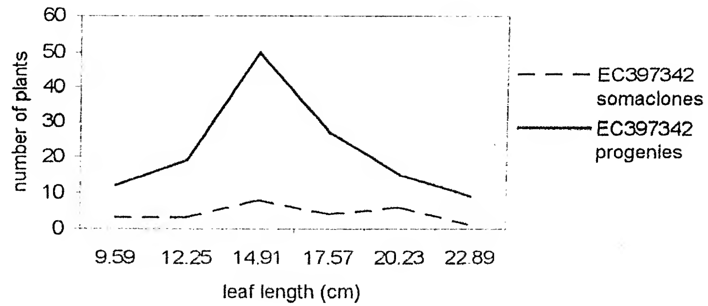
Progenies tended to stabilized around the mode of distribution and exhibited less frequency of variants at the downward side of the distribution of leaf width compared to that somaclones, in *C.setigerus* genotype EC400639. Most



C.ciliaris



C.setigerus



C.echinatus

Fig 6 : Frequency distribution of somaclonal variation for leaf length in somaclones and their progenies

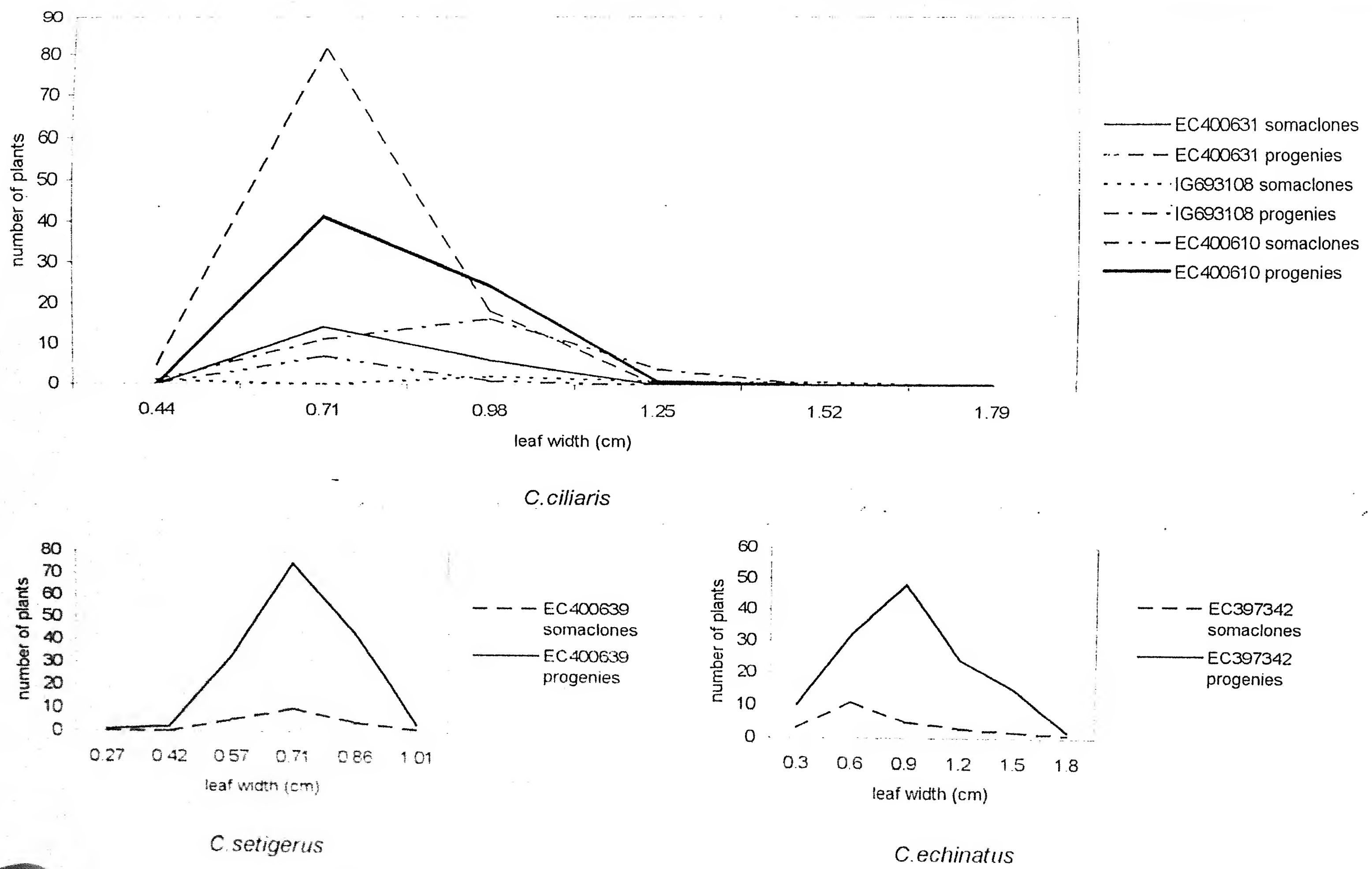


Fig 7 Frequency distribution of somaclonal variation for leaf width in somaclones and their progenies

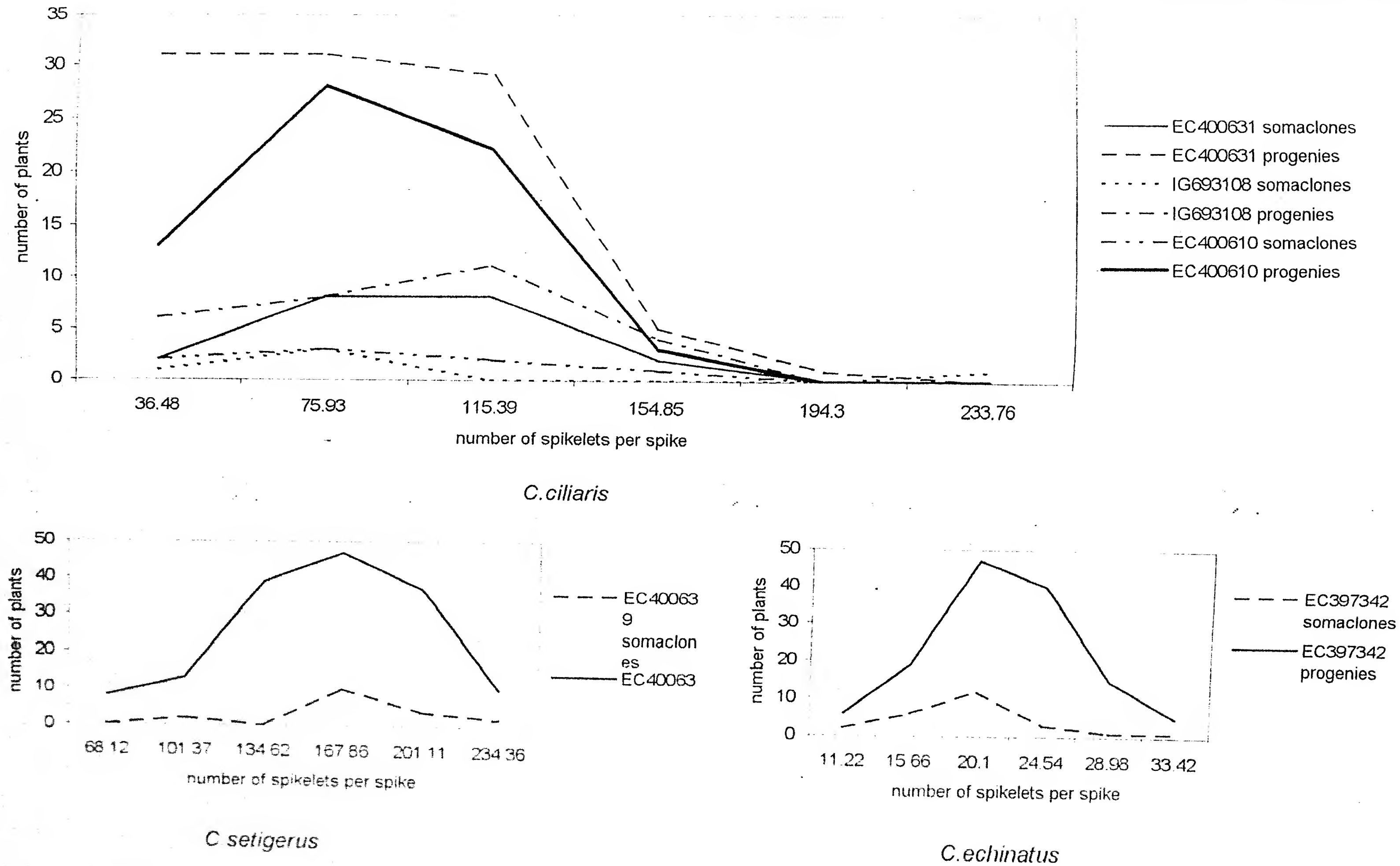


Fig 9 . Frequency distribution of somaclonal variation for number of spikelets per spike in somaclones and their progenies

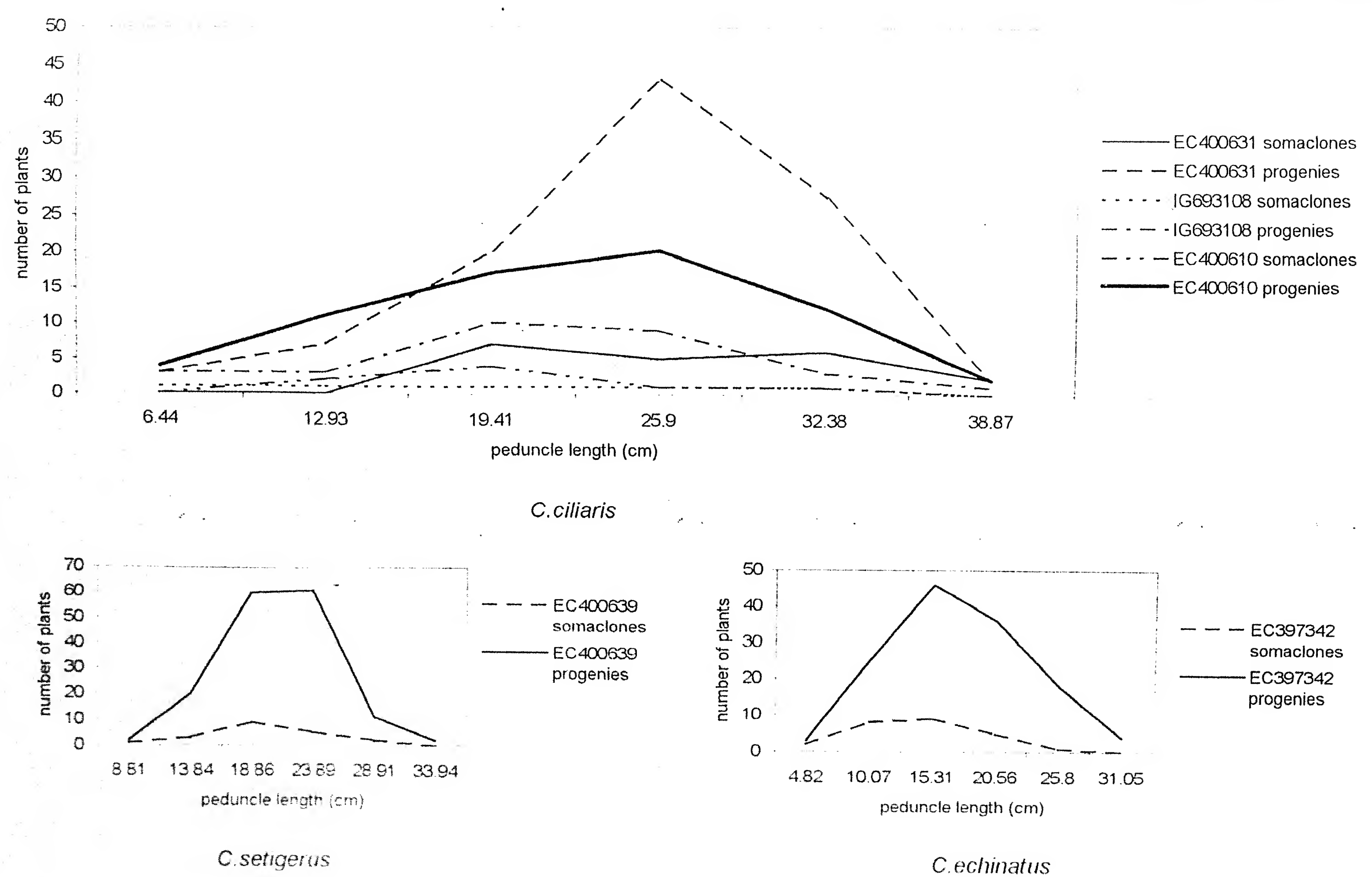


Fig 10 Frequency distribution of somaclonal variation for peduncle length in somaclones and their progenies

contrasting trend was observed in the *C.echinatus* genotype EC397342 where a clear shift in the mode of the progeny distribution was noticed with more progenies being represented at higher leaf width (fig.7).

- **Internodal length**

The type of distribution was similar in somaclones and progenies of genotype EC400631 for internodal length (fig.8). Genotype EC400610 also recorded similar distribution of variation for internodal length and its mode in both somaclones and progenies. However, highly contrasting type of distribution was recorded for the internodal length of *C.ciliaris* genotype IG693108 with shifting of progenies at upward side.

In case of *C.setigerus* and *C.echinatus*, there was clear indication of difference in mode of distribution for internodal length in somaclones and progenies.

- **Number of spikelets per spike**

Somaclones of all genotypes of *C.ciliaris* were different in distribution for number of spikelets per spike (fig.9) and its mode from their progenies however, frequency was less at higher classes in all cases except in the somaclones of IG693108.

Most contrasting trend was observed in the *C.setigerus* genotype EC400639 with bimodal distribution of variation of somaclones. *C.echinatus* also exhibited different mode of distribution for number of spikelets per spike in somaclones and progenies (fig.9).

- **Peduncle length**

Mode of distribution was highly contrasting in the somaclones and progenies of EC400631 (fig.10) with bimodal distribution of variation for peduncle length in somaclones. In case of IG693108, progenies exhibited skewed and asymmetrical mode of distribution compared to that of somaclones and exhibited less frequency at higher frequency class. Entirely different type of distribution with an upward shift in the mode of the progenies was recorded for the peduncle length of *C.ciliaris* genotype EC400610.

The progenies of *C.setigerus* recorded skewed mode of distribution comapared to that of somaclones. The type of distribution was also different in case of

C.echinatus with lesser frequency of somaclones at higher peduncle length (fig.10).

- **Spike length**

Among the *C.ciliaris* genotypes, EC400631 showed very different distribution of variation for spike length and its mode with bimodal distribution of progenies. Two adjacent frequency classes contained almost equal number of progenies. Different type of distribution with upward shift in the mode of progenies was noticed for the spike length in IG693108. Though the lesser frequency was observed at higher spike length. Somaclones and progenies of genotype EC400610, there was considerable difference in mode of distribution between them (fig.11).

Very similar distribution variation for spike length and its mode in somaclones and progenies was found for *C.setigerus* genotype EC400639 with more variants being represented at higher spike length in both. However, highly contrasting mode of distribution was noticed in *C.echinatus* genotype EC397342 with skewed distribution of progenies (fig.11)

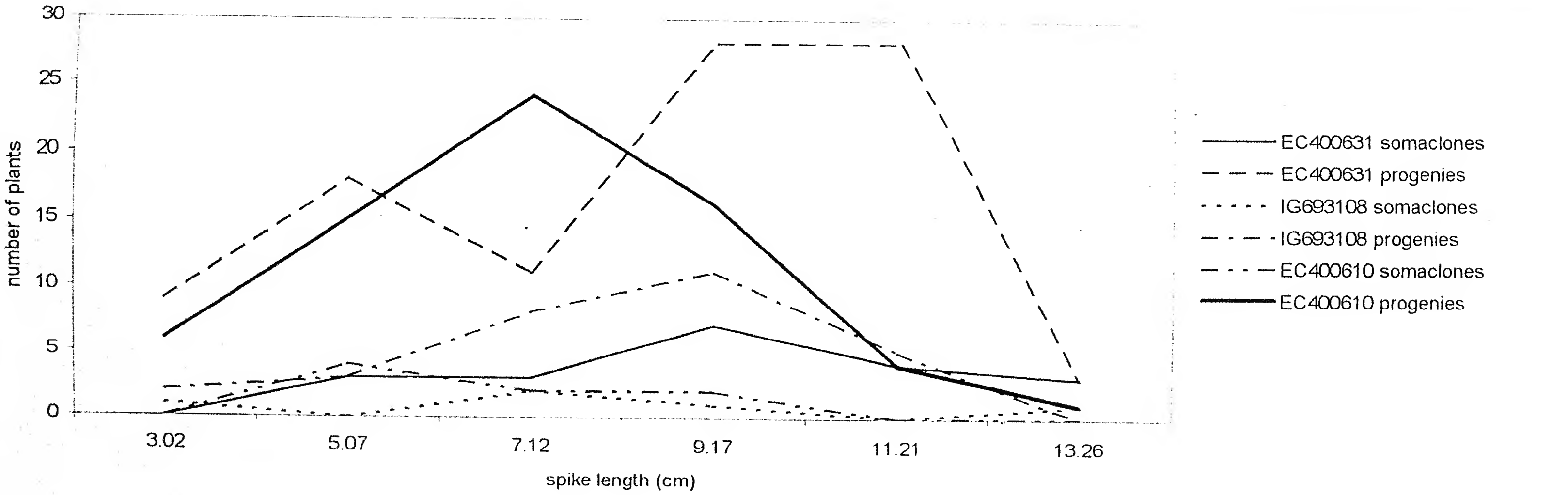
- **Spike width**

Similar trend was observed for spike width and its mode in the genotype EC400631 for the distribution of somaclones and progenies, while in the case of EC400610, different type of distribution of variants with unimodal and asymmetric distribution of progenies were recorded for spike width. Highly contrasting trend was noticed in the genotype IG693108, where a clear shift in the mode of progeny distribution was noticed (fig.12).

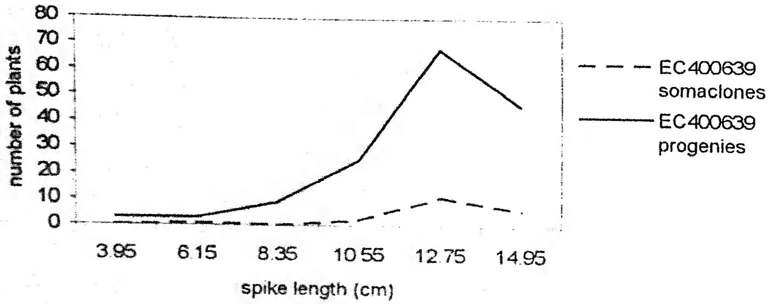
The mode of distribution of spike width was quite similar in somaclones and progenies in *C.setigerus* genotype EC400639, more variants being represented by higher spike width. While *C.echinatus* genotype EC397342 exhibited different mode with skewed distribution of progenies (fig.12).

- **Hundred bur weight**

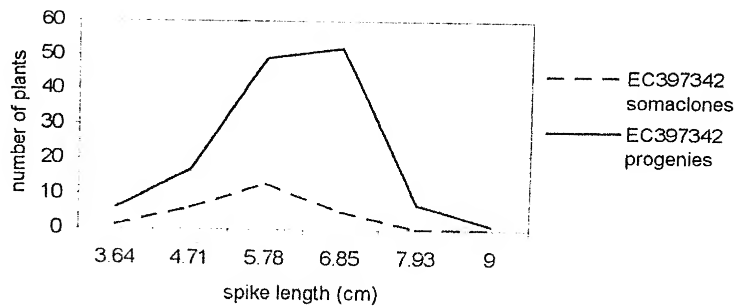
Similar distribution of variation for hundred bur weight and its mode in both somaclones and progenies were recorded in EC400631 (fig.13). Progenies tended to stabilize around the mode of distribution. In case of EC400610, the type of distribution was almost similar in somaclones and progenies. The mode



C.ciliaris

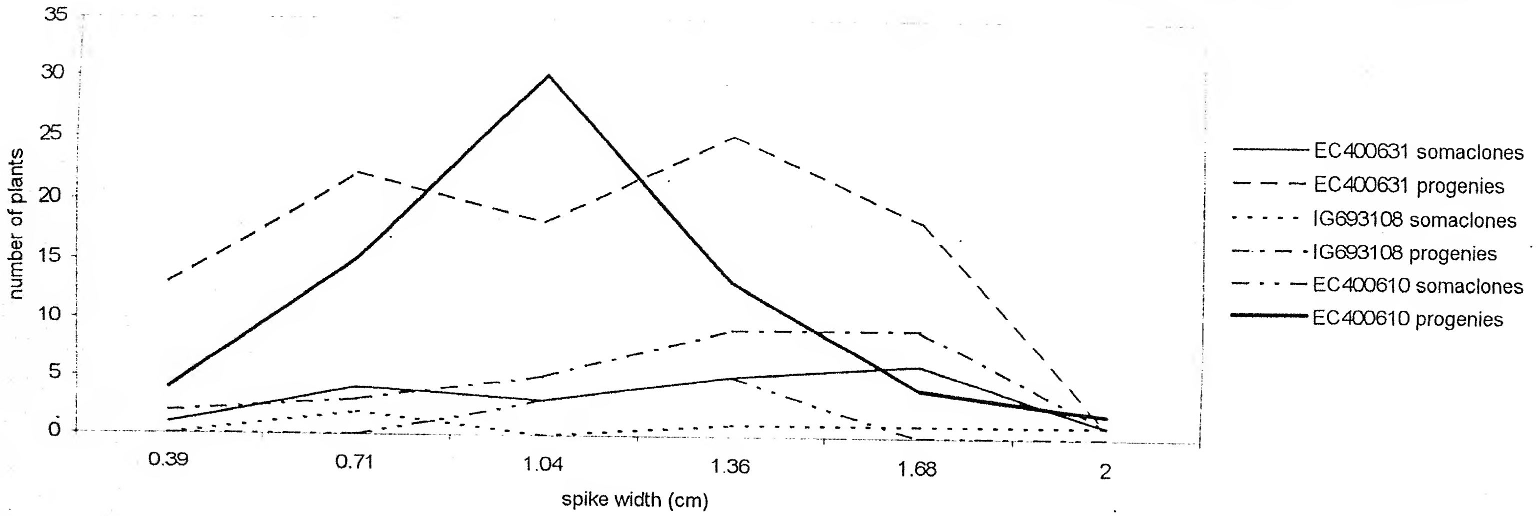


C.setigerus

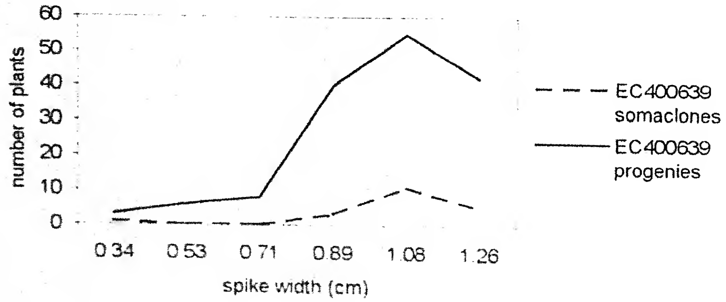


C.echinatus

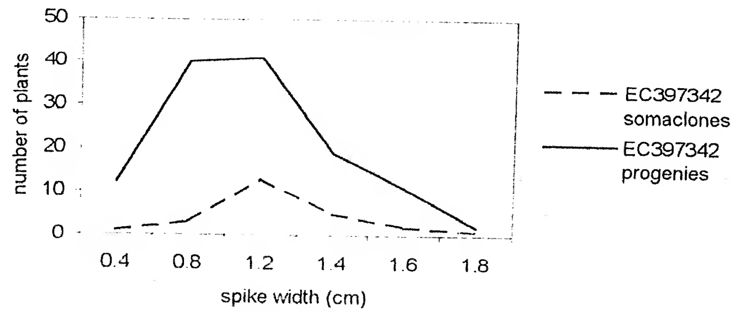
Fig 11 : Frequency distribution of somaclonal variation for spike length in somaclones and their progenies



C. ciliaris

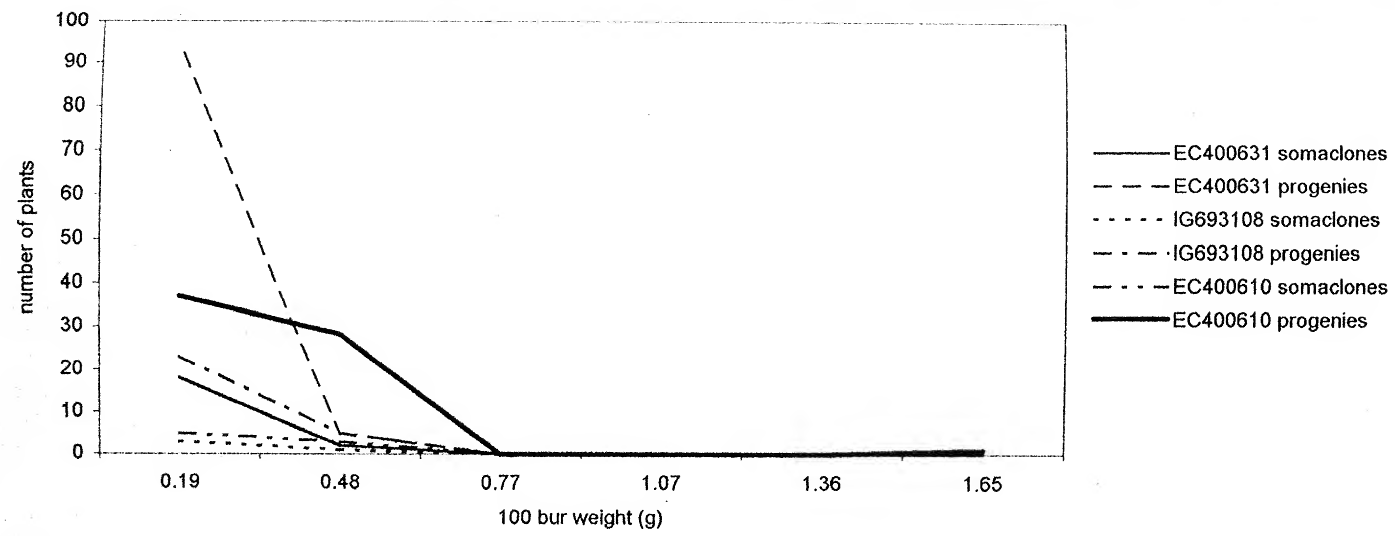


C. setigerus

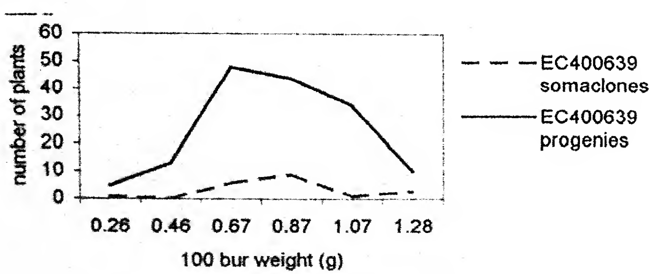


C. echinatus

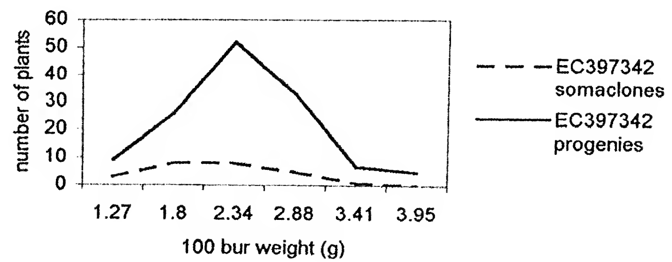
Fig 12 : Frequency distribution of somaclonal variation for spike width in somaclones and their progenies



C.ciliaris

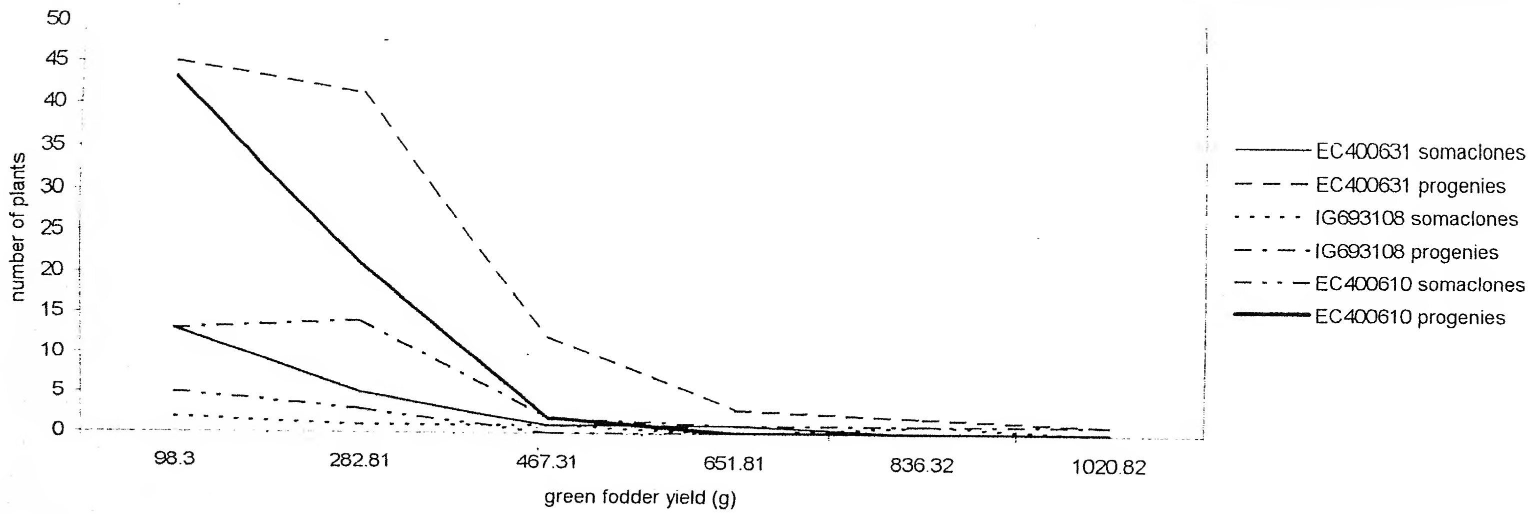


C.setigerus

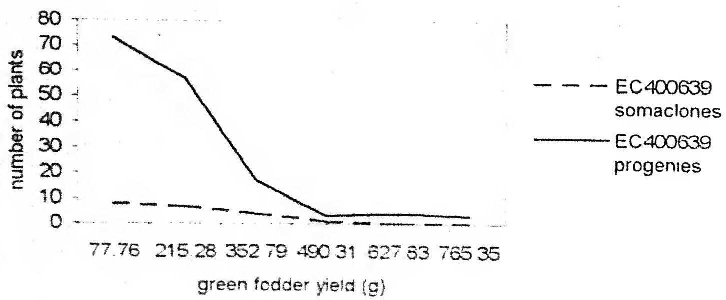


C.echinatus

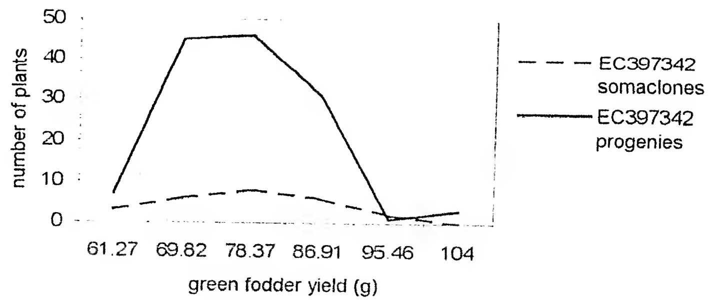
Fig 13 : Frequency distribution of somaclonal variation for 100 bur weight in somaclones and their progenies



C.ciliaris

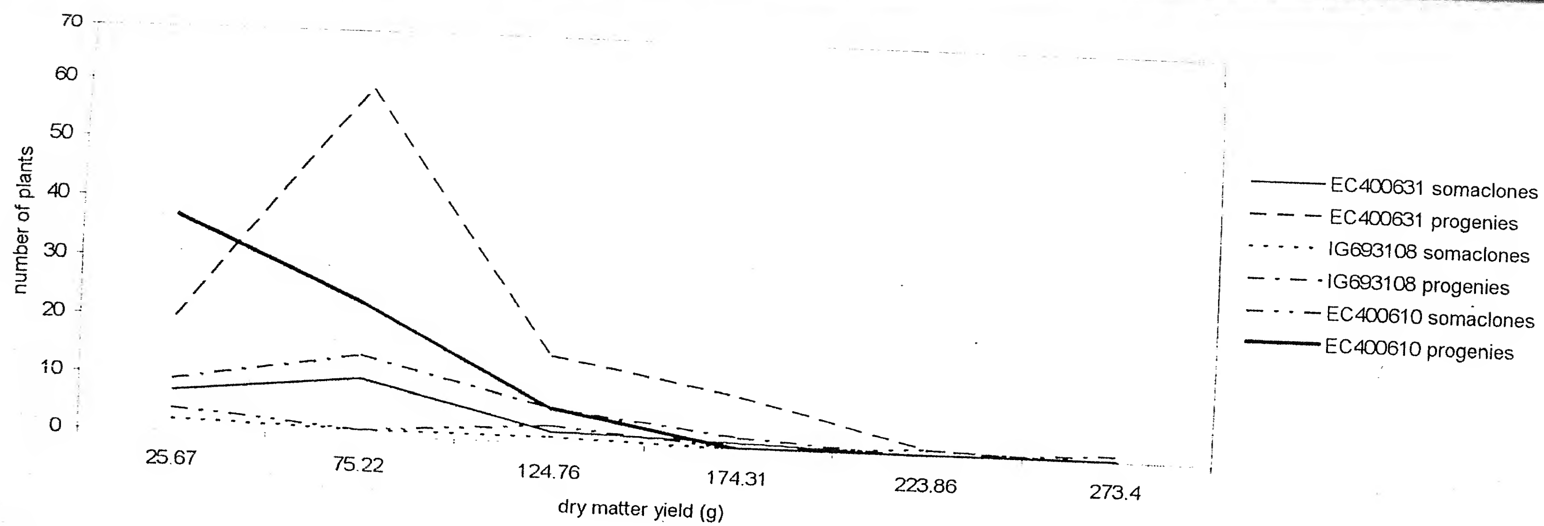


C.setigerus

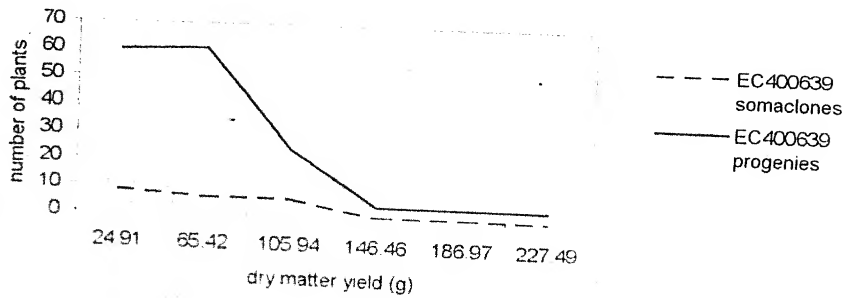


C.echinatus

Fig14 : Frequency distribution of somaclonal variation for green fodder yield in somaclones and their progenies



C.ciliaris



C.setigerus

Fig 15 : Frequency distribution of somaclonal variation for dry matter yield in somaclones and their progenies

of distribution for 100 bur weight was quite similar in somaclones and progenies of *C.ciliaris* genotype IG693108.

Different type of distribution with downward shift in the mode of progenies was recorded for 100 bur weight of *C.setigerus* genotype EC400639 compared to that of somaclones. Progenies were stabilized around the mode of distribution and exhibited less frequency at the upward side of the ftrequency class of 100 bur weight as compared to that of somaclones in *C.echinatus* genotype EC397342 (fig.13).

- **Green fodder yield (GFY)**

Among the *C.ciliaris* genotypes, EC400631 and EC400610 exhibited very similar distribution of variation for GFY (fig.14) and its mode in somaclones and progenies. However, different type of distribution with downward shift in the mode of progenies was recorded for the GFY of *C.ciliaris* genotype IG639108.

Progenies did not seem to be stabilized around the mode of distribution and exhibited less frequency at the higher GFY compared to that of somaclones in *C.setigerus* genotype EC400639. Most contrasting trend was observed in the *C.echinatus* genotype EC397342 where almost equal distribution for GFY was found in two adjacent frequency classes (fig.14)

- **Dry matter yield**

Genotype EC400631, exhibited similar distribution for DMY and its mode in both somaclones and progenies. Progenies crowded around the mode indicating stabilization of variation. In case of EC400610, frequency distribution was almost similar in somaclones and progenies. However, for IG693108, different type of distribution was noticed for progenies and somaclones (fig.15).

Progenies of *C.setigerus* were asymmetric in distribution for dry matter yield compared to that of somaclones. However, both represented lesser frequency at higher dry matter yield (fig.15).

4.2.2. Isozyme analysis

(a) Isozyme Pattern

Evaluation of somaclones through isozyme analysis revealed marked variation among the somaclones with respect to their parent (Table 4.54) (Plate # 18, fig.1,2,3). Total eleven somaclones of different explant (seed and immature inflorescence) of the genotype EG400631 (100000) were evaluated. Percentage of somaclones differing from parent ranged between 0-100% from different isozymes. EST, PGM, PGI and ACP indicated all somaclones to be different from the parent for both seed and immature inflorescence explant derived somaclones while SOD indicated 0 and 55.5% somaclonal variation for seed and immature inflorescence explant derived somaclones, respectively. G6PDH was almost similar for both the explant (50 and 44.4%). Maximum two electrophoretic phenotypes (EPPs) were observed in somaclones derived from seed explant. One EPP each was found for SOD and PGI enzymes, while immature inflorescence explant somaclones showed the range of EPPs between 3-8 for the isozyme PGM and PGI followed by SOD and ACP (5 EPPs) while least number of EPPs were present in EST (3 EPPs) followed by the G6PDH (4 EPPs).

Two somaclones of genotypes EC397600 (200000) were assayed. Both somaclones differed from parent for all the enzymes assayed. Maximum number of two EPPS were recorded for EST and SOD.

With regard to genotype EC400587 (300000), all somaclones differed from the parent for the enzymes EST, PGM, PGI and G6PDH, where as similar banding pattern as that parent for SOD and ACP.

Three somaclones of the genotype IG693108 (400000) were assayed. All enzymes indicated 100% difference between parent and somaclones except SOD. Maximum three EPPs were recorded for the enzyme PGM, PGI and G6PDH followed by two EPPs for EST enzyme.

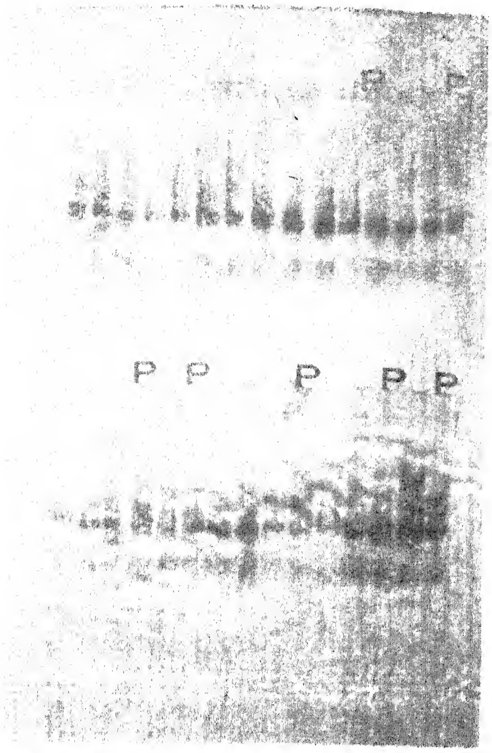
The percentage of somaclones of genotype EC400610 (500000) differing from parent with respect to different isozymes varied between 66.6-100%. Number of EPPs for different enzymes were ranged between 2-10. Highest was observed for the enzyme EST (10) followed byt the enzyme PGM and ACP (8), while lowest number of EPP was recorded for the enzyme SOD (2).

Table 4.54: Zymogram analysis among the parents and their somaclones in *C.ciliaris* and *C.setigerus*

Cenchrus ciliaris	EST	SOD	PGM	PGI	G6PDH	ACP
Parent EC400631(100000)						
Number of somaclones assayed	2	2	2	2	2	2
Total number of bands	13	3	13	10	7	10
Number of parents in the band	9	2	8	2	0	3
Number of polymorphic bands	7	0	4	1	5	2
Number of electrophoretic phenotypes in somaclones	2	1	2	1	2	2
Number of somaclones similar to parent	0	2	0	0	1	0
Number of somaclones differing from parent	2	0	2	2	1	2
Percentage of somaclones differing from parent (%)	100	0	100	100	50	100
Parent EC400631(100000)						
Number of somaclones assayed	9	9	9	9	9	9
Total number of bands	13	3	13	10	7	10
Number of parents in the band	9	2	8	2	0	3
Number of polymorphic bands	7	3	9	8	4	6
Number of electrophoretic phenotypes in somaclones	3	5	8	8	4	5
Number of somaclones similar to parent	0	4	0	0	5	0
Number of somaclones differing from parent	9	5	9	9	4	9
Percentage of somaclones differing from parent (%)	100	55.5	100	100	44.4	100
Parent EC397600 (200000) Explant seed						
Number of somaclones assayed	2	2	2	2	2	2
Total number of bands	13	3	13	10	7	10
Number of parents in the band	9	2	3	2	1	4
Number of polymorphic bands	1	1	3	5	4	4
Number of electrophoretic phenotypes in somaclones	1	1	2	2	2	2
Number of somaclones similar to parent	0	0	0	0	0	0
Number of somaclones differing from parent	2	2	2	2	2	2
Percentage of somaclones differing from parent (%)	100	100	100	100	100	100
Parent EC400587 (300000) Explant seed						
Number of somaclones assayed	1	1	1	1	1	1
Total number of bands	13	3	13	10	7	10
Number of parents in the band	9	2	7	0	0	5
Number of polymorphic bands	1	0	4	1	1	1
Number of electrophoretic phenotypes in somaclones	1	1	1	1	1	1
Number of somaclones similar to parent	0	1	0	0	0	1
Number of somaclones differing from parent	1	0	1	1	1	0
Percentage of somaclones differing from parent (%)	100	0	100	100	100	0

Plate # 18

Polyacrylamide gel electrophoresis



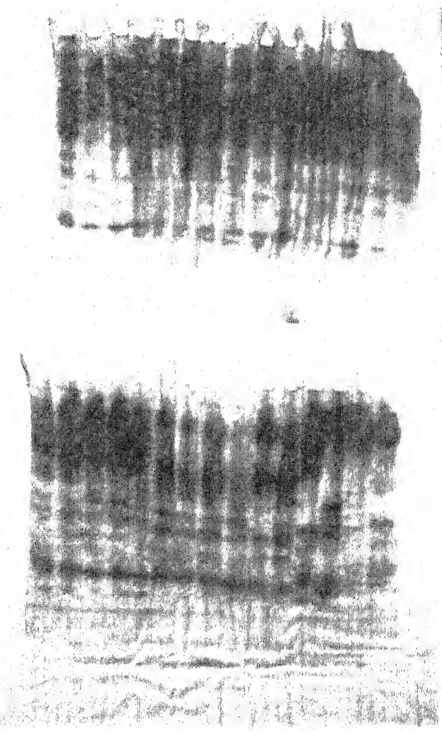


fig-1 Phosphoglcomutase fig-2 Esterase

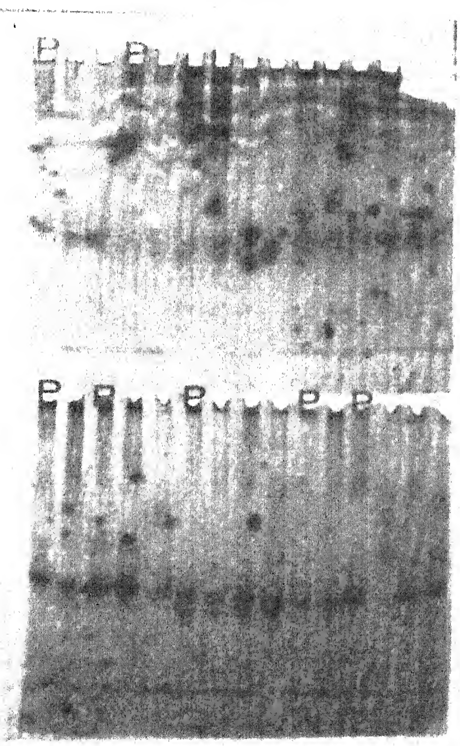


fig-3 Acid phosphatase

P- denotes the Parent and adjacent to it, respective somaclones

Percentage of somaclones of genotype EC397680 (600000) differing from parent was found between the range of 50-100%. Two EPPs were recorded for all the isozymes.

C.setigerus, genotype EC400639 (700000) showed the percentage of differing somaclones between 0-100% on assaying different isozymes. Somaclones differed 100% from the parent for the enzymes SOD, PGM, PGI and ACP. Only one EPP was found for all the enzymes. However, number of polymorphic bands ranged between 1-6.

(b) Dendrogram analysis

Dendrogram analysis indicated six broad clusters consisting of seven parts and their somaclones (fig.16). Fifth cluster consisted of maximum number of genotypes [7 *viz.* genotype EC400587 (300000) and its somaclone (310100), three somaclones (410200,410500,410400) of genotype IG693108 (400000) and one somaclone (510900) of genotype EC400610]. Fourth cluster contained least number of genotypes [3 *viz.* genotype EC400610 (500000) and its two somaclones (510500, 511200)]. Cluster one contained four genotypes [genotype EC400631 (100000) and its three somaclones (110100, 110200, 121300)], second cluster contained six genotypes [four somaclones (120100,120900,121200,121600) of genotype EC400631 (100000) and genotype EC397680 (600000) with its somaclone (610100)] third contained five genotypes [four somaclones (121500, 121400.121100.121800) of genotype EC400631 (100000) and one somaclone (610200) of genotype EC397680 (600000) and sixth cluster consisted of five genotypes [genotype EC397600 (200000) and its two somaclones (210200, 210100) and *C.setigerus* (700000) and its somaclone (711000)].

Genotype EC400631 was genetically most distant from genotype EC397600. Somaclones of genotype EC400631 (100000) were distributed in three clusters. Among them three somaclones (110200,110100,121300) were found in the first cluster along with their parent. Second cluster contained four immature inflorescence derived somaclones (120900, 120100, 121200, 121600) of this genotype along with the genotype EC397680 (600000) and one of its somaclone (610600). Four immature inflorescence derived somaclones (121500, 121400, 121100, 121800) of the genotype EC400631 (100000) were distributed in third

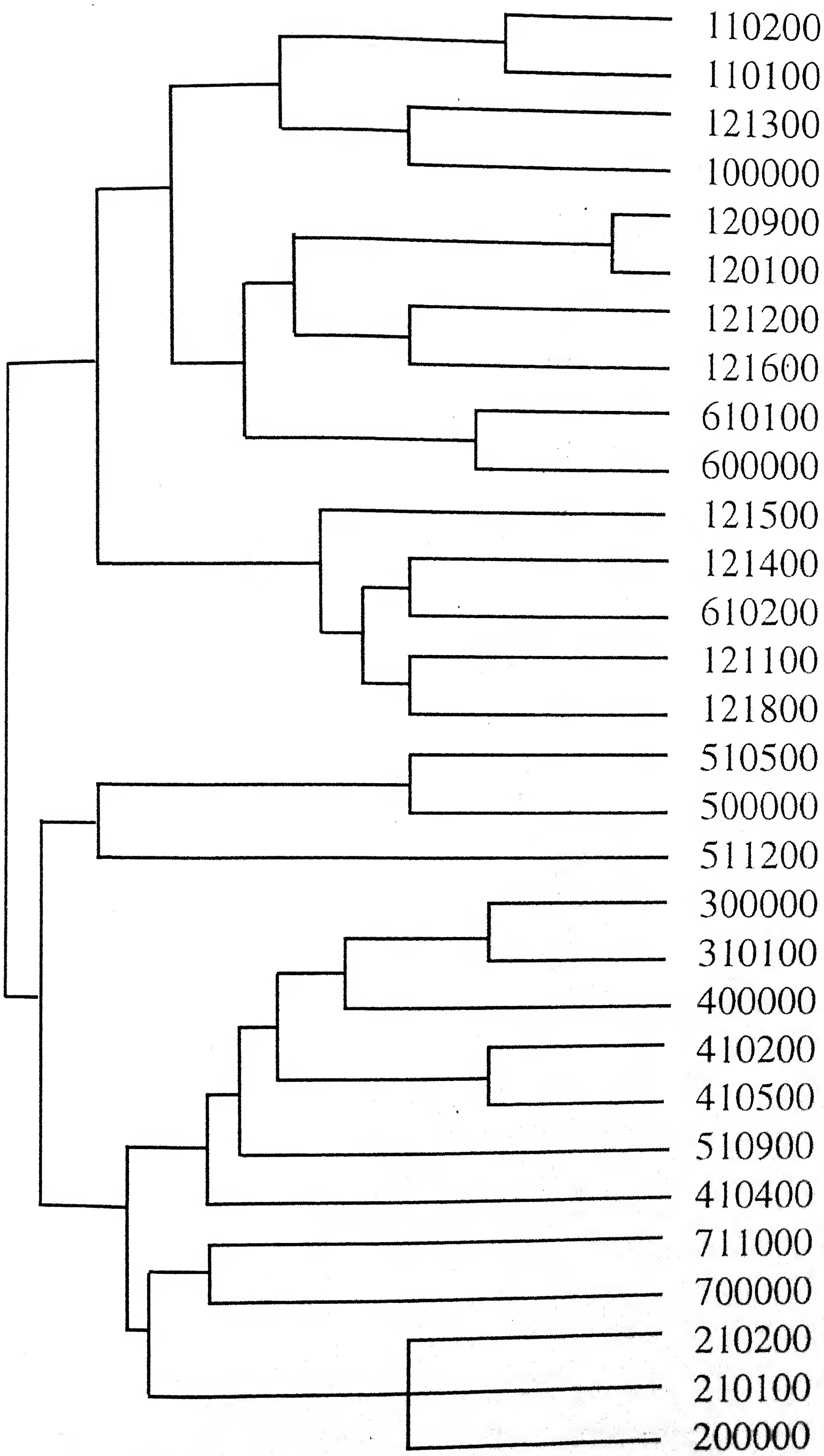


Fig-16: Dendrogram of the somaclones and their parents by isozyme analysis

cluster. One somaclone (610200) of the genotype EC397680 (600000) was also found among these somaclones.

Although the somaclones of genoptype IG693108 (400000) were distributed in the same cluster two of its somaclones (410200, 410500) were nearer (78.6%, 73.2%) than the third one (410400) with the similarity of 71.4% .

Genotype EC400610 (500000) and its somaclones were distributed in two clusters (fourth and fifth). Somaclone 510500 was genetically more similar (76.8%) than somaclone 511200 (64.3%) to their parent, where as one somaclones 510900 was found closer to the somaclones of genotype IG693108 (400000) in fifth cluster.

Accordingly to the dendrogram the genotype EC397680 (600000) was placed along with one of its somaclone (610100) in second cluster. Another somaclone (610200) was placed distantly from its parent in third cluster having the similarity of 75.0% with its parent.

EC400639 (700000) belonging to *C.setigerus* was placed with genotype EC397600 (200000) of *C.ciliaris* in sixth cluster. Dendrogram showed that parent and its somaclone were genetically similar (69.6%).

4.2.3 Chemical Analysis

Bidirectional variations were observed for fodder quality traits in the somaclones and their progenies in most of the evaluated and tested genotypes (Table 4.55).

Genotype EC400631 (100000) showed 1.84% crude protein. Among somaclones, CP content varied between 2.56 to 3.38% however, mean CP content was 3.05 in somaclones. In progenies, CP content increased from 3.10 to 3.85%. Parent had 79.13% NDF content, while mean concentration of NDF in somaclones was 76.47%. Maximum and minimum NDF content were observed in somaclone No. 121400 (80.31%) and somaclone No.121700 (76.00%) respectively. Progenies did not show wide variability in NDF contents. Parent had higher ADF (54.25%) then the mean content of somaclones (44.96%) and progenies (43.65%), respectively. Similarly the contents of cellulose and lignin were more in parent (42.2 and 6.68%) than the somaclones (33.29 and 6.31%) and progenies (34.15 and 6.77%), respectively. Parent contained 24.88% hemicellulose while somaclone No. 121100 and 120300 showed highest hemicellulose content (35.94%) and lowest (25.06%)

hemicellulose content, respectively. Somaclones had higher (31.51%) amount of hemicellulose then its parent. Parent indicated lower AI (67.12%) than the mean value in somaclones (72.2%). AI values of progenies varied between 72.04-75.08%.

Genotype EC397600 (200000) contained higher CP 4.81% than its somaclone (2.95%) and progenies (3.25%). NDF was also more in this parent (77.03%) than its somaclone (76.45%) and progenies (75.3%). However, ADF was found more in somaclones (42.26%) than its parent (42.08%) and progenies (41.12%). Cellulose content was also higher in somaclone (34.74%) than the parent (27.88%) and progenies (29.97%). Lignin content of parent 6.69% was nearly double to that of somaclones (3.73%). Hemicellulose per cent in parent (34.35%), somaclone (34.19%) and progenies (34.18%) were comparable. AI value pf parent (70.52%) was lower than somaclone (84.16%) and progenies (81.16%) respectively.

Parental genotype EC400587 (300000) contained 3.5% CP that was close to its somaclone (3.47%) but lower than the progenies (4.33%). Decreasing value for NDF was observed in progenies. Progenies showed lower ADF 40.42 % content than somaclone (48.25%) and the parent (49.78%). Cellulose content was higher in parent (39.04%) than its somaclone (32.93%) and progenies (33.68%). Lignin was observed higher in parent (9.95%) than its somaclone (7.5%) and progenies (6.02%0. AI value was 62.9%, 54.7% and 73.48% for parent, somaclone (R_0) and progenies (R_1) respectively.

In genotype IG693108 (400000) parent was identical in CP (3.5%) to mean value of its somaclones (3.5%) and progenies (3.57%). NDF accumulation was less in parent (74.84%) than mean concentration of its somaclones (76.12%) and progenies (75.99%). However, ADF content was more in parent (50.99%) than the somaclones (48.09%) and progenies (47.95%). Lignin was lower in parent (6.87%) as compared to its somaclones (7.68%) and progenies (8.34%). Somaclones and progenies had higher hemicellulose contents compared to the parent. AI value of the parent (72.69%) were more than its somaclone (67.44%) and progenies (64.12%).

In the genotype EC400610 (500000), the CP content was more in parent (4.99%), than its somaclone (4.04) and progenies (4.05). Parent showed lower NDF content

Table 4.55: Comparative values of CP, fiber fraction and AI values in the parent, somaclones and progenies of *Cenchrus* species

	Crude protein (CP%)	Neutral Detergent Fibre (NDF%)	Acid Detergent Fibre (ADF%)	Cellulose %	Lignin %	Hemicellulose %	Availability Index %
EC400631 (100000)	1.84	79.13	54.25	42.2	6.86	24.88	67.12
120300	2.56	76.42	51.36	36.73	6.7	25.06	71.58
120301	3.1	74.07	54.12	40	6.46	19.95	75.08
121400	3.38	80.31	45.23	33.9	6.85	35.08	65.21
1214001	4.31	72.42	42.76	33.37	6.39	29.66	76.83
1214002	3.32	74.39	44.54	34.93	7.16	29.85	72.04
Average progeny 1 & 2	3.38	73.4	43.65	34.15	6.77	29.75	74.43
121700	3.34	76	46.04	33.67	6.73	29.96	71.95
12100	2.92	73.17	37.23	28.89	5.34	35.94	80.09
Average somaclone	3.05	76.47	44.96	33.29	6.31	31.51	72.2
EC397600 (200000)	4.61	77.03	42.06	27.88	6.69	34.35	70.52
210200	2.95	76.05	42.26	34.74	3.73	34.19	84.16
210203	3.14	76.09	39.99	29.31	5	36.1	79.08
210205	3.36	74.52	42.26	3.65	4.31	32.26	83.08
Average progeny 3 & 5	3.25	75.3	41.12	29.98	4.65	34.18	81.08
EC400587 (300000)	3.5	79.78	49.78	39.04	7.5	30	62.9
310100	3.47	78.03	48325	32.93	9.95	29.78	54.7
310103	3.53	74	4093	28.32	4.54	31.66	84.02
310105	5.14	71.58	39.92	39.04	7.5	30	62.9
Average progeny 3 & 5	4.33	72.79	40.42	33.68	6.02	30.83	73.48
IG693108 (400000)	3.5	74.84	50.99	37.58	6.87	23.85	72.69
410400	2.95	73.82	48.52	37.49	7.45	25.3	71.54
410402	4.04	79.42	50.59	37.25	8.59	28.83	58.26
410500	4.06	78.42	47.67	37.86	7.91	30.75	63.34
410501	3.12	78.01	48.69	33.63	11.2	29.32	49.06
410502	4.99	77.62	47.35	37.01	6.75	30.27	69.83

Contd. table 4.55

	Crude protein (CP%)	Neutral Detergent Fibre (NDF%)	Acid Detergent Fibre (ADF%)	Cellulose %	Lignin %	Hemicellulose %	Availability Index %
410503	3.82	72.35	47.81	36.31	7.08	24.54	74.39
Average progeny 12 & 3	3.57	75.99	47.95	35.65	8.34	28.04	64.42
Average somaclone	3.5	76.12	48.09	37.67	7.68	28.02	67.44
EC400610 (500000)	4.9	67.73	44.49	32.84	5.38	23.21	83.32
510600	2.76	75.94	48.87	37.03	7.69	27.07	68.03
510601	4.23	79.75	49.68	36.01	7.85	30.07	61.23
510900	5.33	74.76	39.56	31.03	4.16	35.2	83.51
510901	4.77	76.1	38	32.1	4.03	38.1	83.13
510902	3.34	74.04	41.83	31.45	6.22	32.21	76.04
Average progeny	4.05	75.07	39.91	31.77	5.12	35.15	79.58
Average somaclone	4.04	75.35	44.21	34.03	5.92	31.13	75.77
EC397680 (600000)	2.57	77.05	52.32	40.65	7.24	24.73	68.45
610200	2.61	80.31	43.57	34.49	6.35	36.73	67.76
610201	3.46	74.63	45.25	32.83	3.4	29.38	85.99
610202	2.57	75.72	45.35	36.72	6.19	30.37	73.71
Average progeny 1 & 2	3.01	75.17	45.3	34.77	4.79	29.87	79.85
EC400639 (700000)	4.28	72.9	41.13	33.72	4.54	31.77	83.7
710700	6.08	73.51	37.4	29.95	3.69	36.11	86.01
710701	5.12	74.33	37.23	28.89	5.34	37.1	79.19
710702	5.36	70.12	37.95	29.19	4.6	32.17	84.56
Average progeny 1 & 2	5.24	72.22	37.59	29.04	4.97	34.63	81.87
710800	5.36	72.08	39.8	33.1	4.67	32.17	84.56
710801	5.92	73.76	35.99	28.21	5.04	37.77	80.79
710802	6.24	70.04	35.46	27.53	5.2	34.58	82.65
Average progeny 1 & 2	6.08	71.9	35.72	27.87	5.12	36.17	81.72
711100	7.04	74.03	43.15	34.08	5.54	30.88	82.65

Contd. Table 4.5[illegible]

	Crude protein (CP)	Neutral Detergent Fibre (NDF)	Acid Detergent Fibre (ADF)	Cellulose	Lignin	Hemicellulose	Availability Index
711200	6.1	81.45	42.2	31.79	5.91	39.25	68.14
711204	3.42	76.75	42.25	33.03	5.51	34.51	77.84
711205	6.15	79.73	42.85	32.43	5.54	36.88	72.66
Average progeny 1 & 5	4.78	78.25	42.55	32.73	5.52	35.69	75.25
711500	4.68	85.98	38.68	30.17	4.8	47.3	65.76
711501	7.51	71.98	37.51	29.8	4.13	34.39	85.26
711502	3.69	77.5	37.33	29.6	4.76	36.85	78.84
Average progeny 1 & 2	5.65	74.74	37.42	29.7	4.44	35.62	82.04
711900	4.12	75.72	37.13	29.83	3.31	38.59	86.36
711901	5.78	67.6	37.67	29.82	4.21	30.53	87
711902	3.77	69.6	39.07	30.58	4.16	30.89	86.15
Average progeny 1&2	4.77	68.78	38.37	30.2	4.18	30.71	86.57
Average somaclones	5.56	77.12	39.74	31.48	4.65	37.38	78.91

(67.73%) than the somaclones (75.35%) and progenies (75.07%). However, ADF contents were more in parent (44.49%) than the mean of its somaclones (44.21%) and progenies (39.9%). Cellulose contents was 32.84% in parent which was less and more than its somaclones (34.03%) and progenies (31.77%), respectively. Lignin content were comparable amongst parent (5.38%), somaclones (5.92%) and progenies (5.12). However, hemicellulose content was lower in parent than its progenies and somaclones. AI was more (83.32%) in parent as compared to its somaclones (75.77%) and progenies (79.58%).

Genotrype EC397680 (600000) showed less CP content (2.57%) than its somaclones (2.61%) and progenies (3.01%). This indicated a gradual increase in CP content from parent to progenies. Higher NDF content was found in somaclone (80.31%) followed by its parent (77.05%) and progenies (75.17%). Less ADF content was found in somaclone (43.57%) than its progeny (45.3%) and parent (52.32%). Cellulose content was also found higher in parent (40.65%), vis-a-vis its somaclone (34.49) and progenies (34.77), resperctively. Lignin was observed higher in parent (7.24%) followed by its somaclone (6.35%). Its progenies contained only 4.79% lignin, which showed decreasing trend. Parent contained lower level of hemicellulose (24.73%) as compare to its somaclone (36.73%) and progenies (29.87%).AI value were recorded 68.45%, 67.76% and 79.85% for parent, somaclone and progenies, respectively.

C.setigerus genotype EC400639 (700000) had 4.28% CP whereas it ranged from 4.12 to 7.04% among somaclones. Average of CP value of somaclones was higher than the parent. Progenies (R_1) of different somaclones showed CP content between 4.77 to 6.08%. The parentral genotype EC400639 (700000) contained 72.95% NDF. Somaclones exhibited NDF in range of 72.08 to 85.98%. Average NDF value of somaclones was 77.12%. Variation ranged between 68.78-78.24%. NDF among progenies. Maximum NDF was observed in progenies of somaclones No. 711500 (74.74%).

Parent showed 41.13% ADF while the average of somaclones was 39.74%. The highest level of ADF was recorded in somaclone No. 711100 (43.15%). Progenies ranged between 35.71 to 42.44% for ADF content. Progenies of somaclone no. 711200 had maximum ADF (42.55) among all progenies of different somaclones.

The parent contained 33.72% cellulose which was more than the mean contents of somaclones (31.48%). The cellulose content ranged from 29.83 to 34.08% in the somaclone. The range of variation for cellulose was between 27.87 and 32.73% in the progenies of somaclones. Highest value was observed in the progenies of somaclones No. 711200 (32.73%) followed by the progenies of somaclone No. 711900 (30.2%).

The parent contained 4.54% lignin, which was lower than the average of somaclones (4.65%). Lignin content varied between 3.31 to 5.91% amongst the somaclones. Maximum lignin was found in somaclone No. 711200 (5.91%) and the maximum lignin content was recorded in the progenies of somaclone No. 711200 (5.52%). Hemicellulose content in parent (31.77%) were lower than meanvalue of its somaclones (37.38%). Progenies showed lesser amount of hemicllulose than their respective somaclone except the progenies of somaclone No. 710800 with 36.17% hemicellulose while its somaclone contained 32.17% hemicellulose. The parent exhibited 83.7% AI. Somaclones ranged between 65.76 to 86.36% for AI with mean value of 78.91%. Highest and lowest AI was recorded for somaclon No. 710900 (86.36%) and somaclonal No. 711500 (65.76%), respectively. Progenies varied between 75.25 to 86.5% for AI. Highest value of AI was recorded in the progenies of somaclone No. 711900 (86.5%) where as lowest value was recorded in progenies of somaclone No. 711200 (72.25%). No definit trend was found for somaclones of *C.setigerus* genotype EC400639 and their progenies with respective chemical constituents.

DISCUSSION

5. Discussion

The genus *Cenchrus* belongs to the tribe Paniceae of Poaceae family and comprises of annual and perennial plants. *C.ciliaris*, commonly known as buffel grass, is a native of tropical and subtropical regions of the old world. In India, it is widely distributed in plains of Rajasthan, Punjab and Western U.P. and is one of the most predominant species of *Dicanthium-Cenchrus-Lesiurus* grass cover of India. It serves as an excellent source of grazing and good quality of hay for live stock in the country. It is highly polymorphic, well adapted to arid and semi-arid areas. It is highly heterozygous, a natural polyploid and represented by several chromosomal races. The tetraploids are the most common representative of the species and are obligate apomicts in their mode of reproduction.

C.setigerus, commonly known as birdwood grass, is a native of Africa, Arabia and India. It is highly palatable, nutritious perennial forage grass, well adapted to semi-arid conditions. It grows well in low rain-fall areas and serves as a stand over feed in these areas. It is also apomictic in its mode of reproduction. *C.echinatus,* commonly known as hedgehog grass, is an annual species. It is distributed throughout the warmer regions of north and south America and Passific Islands. In India, it is found in plains of north-west region. It is much valued as a forage grass on account of early appearance of its foliage. Genetic improvement of these grass species for recombination the desirable traits through conventional breeding methods has been very difficult and is limited upto selection of a few promosing plant types only due to predominantly occurring apomictic mode of reproduction. Hence, there has been a constant need to manipulate their genetic architecture

adopting alternate proceduress, such as, biotechnological tools vis-à-vis selection procedures.

Biotechnology has revolutionized almost all the aspects of biological science in recent past. It has greatly influenced the development in the field of genetics and led to extensive use of biological systems for technological advancements of agricultural production throughout the world in a significant manner. Most of the biotechnological advances in plant science and their use in innovating modern agriculture primarily stamp from their successful use of plant tissue culture systems. The foundation of plant tissue and cell culture was laid down for the first time by Haberlandt (1902) by conceptualizing the phenomenon of "totipotency" of plant cells. However, the establishment of totipotency could be realized only after the discovery of hormonal regulation of growth and differentiation of higher plant cells by Skoog and Miller (1957) followed by the development of experimental procedures for successful cultures of tissues (Reinert, 1959), cell suspension and eventually single cell cultures (Steward *et al.*, 1958) and their differentiation and morphogenesis. Although tissue culture of most of the species of agriculture interest has been possible, however, relatively few such species of the most important group, Gramineae have exhibited reproducible and efficient regeneration systems.

The concept of totipotency has provided the basis of several approaches aiming at inducing genetic modification of the plant cells which would survive through tissue culture and be expressed phenotypically in the whole plant. Plant cell cultures have provided a new and existing option for obtaining increased genetically variability relatively rapidly. The variability generated by the use of tissue culture cycle is referred as **Somaclonal Variation** and the somaclonal variation is now and established phenomenon (Larkin and Scowcroft, 1981). It is evident from a number of studies that genetic changes occur in plant tissue cultures and these changes are transmitted to the regenerated plants and their progenies (Larkin and Scowcroft, 1981 and Shepard, 1981). The utilization of new genetic variability induced during the culture process has now become one of the major objectives of tissue culture. A series of reports on a wide range of genetic variation among regenerated plants from protoplasts, cell and callus cultures have stimulated interest in this type of

variation for the point of view of using it in genetic improvement of various crop species (Shepard *et al.*, 1980; Shepard, 1981; 1982 and Thomas *et al.*, 1982).

Majority of plant traits for which genetic variability is generated during tissue culture cycle are of genomic value and can thus provide valuable adjurnet to plant improvement. Tissue culuture variation is superimposed directly upon existing qualities of cultivars. The heightened interest in somaclonal variations has emerged in part from the fact that modern genetically sophisticated cultivars might be improved by generating new genetic variability within coopted gene complexes in a sequential manner by selection and/or screening among the plants and their progenies regenerated from tissue and cell culture. Much of variation generated during tissue culture has been demonstrated to be heritable in many agronomically important crop plants and its possible value in upgrading the genetic base in valuable varieties.

The increase in genetic variablility through tissue culture was first achieved in sugarcane followed by rice, wheat, lettuce and tomatoes (Larkin and Scowcroft, 1981). The techniques, such as, somaclonal variation might be the only practical way to generate variation in asexually propogated and apomictic species. Among asexually propogated food crops, sugarcane and potato have demonstrated that somaclonal variation could provide extensive and possibly useful phenotypic variability.

In view of this background the present study was conducted in *Cenchrus* genotypes. Since hybridization in *Cenchrus* has been difficult due to the apomictic mode of reproduction and small size of florets, this crop was thought to be a suitable candidate for improvement through somaclonal variation. Hence, the first task was to get somaclones in different genotypes through optimized *in vitro* callus production and regeneration and the second task was to evaluate the somaclones for variation in important agronomic traits and their expression in the SC_1 generation and their progenies.

5.1 Optimization of Callus Induction and Plantlet Regeneration

Basic parameters for the measurement of good callus induction are callus induction frequency and callus regenerability. In the present study, these parameters were measured as per cent explants responding for callus induction and callus quality. Callus induction and plantlet regeneration have been dependent on genotype, explant, medium and culture conditions (Vasil, 1986).

(A) Callus Induction

Mature seeds and immature inflorescence were used as explants and they were put on the callusing medium after proper and uniform sterilization. The observations on callus induction were recorded 20 days after innoculation. Differences with respect to callus induction frequency with respect to genotyopes, growth regulators and media were studied.

(i) Effect of media

The callus induction parameters in the present experiment revealed highly significant differences among MS, SH and B_5 media for both seed and immature inflorescence explants. In all the genotypes of *Cenchrus* species, MS medium proved best for callus induction followed by the SH medium. Highly significant difference for immature inflorescence explant and non-significant differences for seed explant were observed for callus induction frequency among the genotypes. Ito and Abe (1990) found in their study that MS media was 6-8 times better than B_5 and N_6 medium, whereas Kalamani and Ramasamy (1998) observed better callus induction in pearl millet on N_6 media than on MS. Bohorova *et al.* (1995) also proved that N_6 basal media was better than any other media in maize. However Sankhla and Sankhla (1989), Kacker and Shekhawat (1991) and Ross *et al.* (1995) used MS medium for callus induction in *C.ciliaris*. This was probably due to the superiority of medium in containing all the essential elements and organic supplements which were required for good callus induction and development. MS medium promoted

increased callus initiation and provided better callus maintenance than Blayeds, B_5, Norstog and SH media (Henzel *et al.*, 1985).

Lambe *et al.* (1999) found that frequency of friable embryogenic callus is related to the composition of the medium. Same results were recorded in tall fescue by Kasperbauer (1990).

Interaction between media and genotype was significant in case of callus induced from seed explant and it was non-significant for callus induced from immature inflorescence. Callus formation was affected by the interaction of genotype and media and the effect was stastically significant (Bregitzer, 1992). Hassawi *et al.* (1990) and Elwaffa (1999) showed highly significant difference between genotype and medium by analysis of variance.

(ii) Effect of Genotype

Genotypes differed significantly from one another on the same and different media and growth regulators. Callus induction frequency in genotypes EC400610 and EC397600 of *C.ciliaris* was recorded very high on all media combinations followed by the genotype EC397680 from the seed explant. Genotype IG693108 performed poorly irrespective of media and the combinations of growth regulators for both seed and inflorescence explants. *C.setigerus* and *C.echinatus* exhibited moderate callus induction from both the explants. However, *C.echinatus* did not show any induction of callus from immature inflorescence explant on any of the media combinations. With respect to immature inflorescence explant, the genotype EC400631 was found best for initiation of callus followed by EC397600 in *C.ciliaris*. Genotypic differences for callusing of immature inflorescence explant were recorded by Farook and Chander (1989) and Selvi (1990) in finger millet. Genotype has been a crucial factor influencing the probability of successful callus induction and plantlet regeneration in cereals (Dykes and Nabors, 1986 and Bhaskaran and Smith, 1990). Genotype dependent differences have also been observed by various workers in different crops, such as, barley (Brigtzer, 1992), wheat (Mathias, 1989; Elwaffa, 1999; Ahmed, 1999 and Elwaffa and Esmail, 1999), rice (Maheswari and Rangasamy, 1989; Izumi *et al.*, 1997 and Bai *et al.*, 1999), maize (Sat'ya *et al.*, 1988 and Ochesanu *et al.*, 1990), sugarcane (Cheema *et*

al., 1992 and Sheng *et al.*, 1998), guinea grass (Akashi and Adachi, 1990), sorghum (Cai and Butler, 1990; Guo and Liang, 1993 and Bhat and Kuruvinashtti, 1994), oats (Rines and McCoy, 1981 and Bregitzer *et al.*, 1989), pearl millet (Nagarathna *et al.*, 1991). Such genotypic differences seem to be due to the differences in endogenous hormone concentrations in the same explant tissue of different genotypes (Fitch and Moore, 1990). This variation for the ability to form regenerable callus was also heritable in sorghum (Ma *et al.*, 1987).

(iii) Effect of Growth regulators

2,4-D and BA were the growth regulators of choice for callus induction. 2,4-D is also a potential mutagen which might induce maximum somaclonal variation (Karp, 1992). BA in lower concentrations, promotes embryogenic competence in callus as was noticed in sugarcane by Cheema *et al.* (1992) and in *Paspalum* by Cardona and Duncan (1997). The direct effect of these growth regulators significantly differed only with respect to callus induction frequency. 2,4-D has been successfully used for promoting regenerable callus production in majority of the cereals and grasses, *viz.*, wheat (Sears and Deckard, 1982 and Elwaffa, 1999), rice (Maheswaran and Rangasamy, 1989 and Kishor *et al.*, 1999), barley (Sarsenbaev *et al.*, 1988 and Taniguchi *et al.*, 1991) and *Cenchrus* (Sankhla and Sankhla, 1989).

In the present investigation also, the effect of 2,4-D on callus induction frequency was highly significant for both seed and immature inflorescence explants in *C.ciliaris.* 2,4-D, at the concentration of 3-5 mg/l was found to be optimum for enhancing callus induction frequency as well as quality in most of the genotypes. However, this difference was not significant in *C.setigerus* and *C.echinatus.* Any deviation from the optimum concentrations of 2,4-D showed adverse effect on callus quality. With the lower concentration (1 mg/l) or higher concentration (10 mg/l) of 2,4-D, callus induction frequency and the quality, both were deteriorated. This was in variance with the study of Kuusiene and Sliesaravicius (1991). They found high frequency of callus formation at 2,4-D concentration of 10 and 15 mg/l in *Festuca pratensis.*

However in zoyisa grass, 2,4-D @ 1 mg/l was found better than its higher concentrations to produce callus from seed explant (Al-Khayri *et al.*, 1989). Maximum embryogenic callus could be induced on MS medium supplemented with 2,4-D @ 3.0-5.0 mg/l and BA @ 0.2 to 0.5 mg/l from both the explant. Morphological differences of calli have been found in different levels of auxin treatments and among cultivars (Abeysekera, 1992) and frequency of embryogenic callus formation was related to the concentration of plant growth regulators (Dolgykh *et al.*, 1999). However, the callogenic response was stimulated by the low levels of 2,4-D and its frequency was significantly reduced when the 2,4-D level was increased (Cardona and Duncane, 1997). 2,4-D was found optimum in sugarcane @ 3 mg/l by Cuellar (1997).

2,4-D x genotype interactions were not significant for both the explant in *C.ciliaris*. In case of *C.setigerus* and *C.echinatus* since one genotype each was used for this study, 2,4-D and genotype interaction couldn't be worked out. However, varying response of the genotypes in producing embryogenically competent callus with the use of different levels of 2,4-D has been documented (Heyser *et al.*, 1983). Fitch and Moore (1990) proposed that callus characteristics were a function of the auxin and the genotype in sugarcane. In wheat, genotypes differed with respect to callus poliferation with the use of different phytohormone sources and their concentrations (Lazar *et al.*, 1983). Highly significant difference was observed between genotypes and 2,4-D (Elwaffa and Ismail, 1999) when callus was raised from immature embryo explant.

The role of cytokinin in conferring competence to generate callus in cereals has not been very clear. In seed explant, addition of BA gave high callus induction frequency with highly significant difference, however, callus quality was not affected much in case of *C.ciliaris*, where as in case of immature inflorescence explant, the effect of BA was not much distinguishable and showed non-significant difference. For *C.setigerus* and *C.echinatus* there was no significant effect of varying levels of BA on callus induction frequency. In sorghum, incorporation of 0.5 mg/l BA to the leaf sheath culture improved the frerquency of callus induction and type of callus. Enhancement of embryogenic callus

formation with the addition of BA in the medium was observed in barley by Rengel and Jeleska (1986). On the other hand, BA inhibited the formation of compact type of calli in bamboo (Zamora *et al.*, 1988). A combination of auxins and cytokinn was found to be suitable for embryogenic callus initiation in several cultivars of rice (Ling *et al.*, 1983 and Fatokun and Yamada, 1984). The depressive effect of kinetin on callus proliferation was noted in wheat (Lazar *et al.*, 1983).

(iv)Effect of adjuvants

Media adjuvants for complementing unspecified nutrients or growth factors are often of much help in callus induction. Adjuvants like coconut water (5-20%) and casein hydrolysate (500-1000 mg/l) enhanced embryogenic callus growth (Armstrong and Green, 1985). CH has been known to facilitate the formation of embryogenic callus and prevent browning of callus in Gramineae (Luhrs and Lorz, 1987). It may also, therefore, help in regeneration from long-term cultures. Noticeable difference in callus formation was observed when CH @ 500mg/l was used for immature inflorescence explant whereas with seed explant, it was moderate in *C.ciliaris. C.echinatus* also showed high callus induction frequency with CH. However, addition of CH did not affect much in callus induction frequency in *C.setigerus*. Mohanty *et al.* (1985) found that the addition of CH (500 mg/l) yielded white and friable callus in finger millet irrespective of the genotype and explant sources. Browing of callus was prevented by CH (500 mg/l) in finger millet callus (Selvi, 1990). In some cases CH seemed to have no effect in callus induction or quality, as shown by Liu (1993) in case of sugar cane. Larkin (1982) found that CH significantly shortened the log period in the growth of sugarcane suspension cultures, though it did not increase the rate of growth following the lag phase.

(B) Callus Growth

Calli were subcultured every 20 days in order to replenish the growth regulators and the nutrients in the culture. Subcultures were also carried out every 20-24 days

in *Cenchrus* by Sankhla and Sankhla (1989). Among all the different culture media used, MS medium in general was found to be the best for higher callus multiplication as against SH and B_5 media for both seed and inflorescence explants. Redway *et al.* (1990) also found in wheat that callus formation and maintenance on MS medium was significantly higher than any other media tried. Gupta *et al.* (2002) observed in sorghum that culture medium had significant effect on callus growth. MS medium was found significantly superior over SH and N_6 media for callus growth. The better callus growth on MS medium than that on N_6 medium is also supported by the observations of Kaeppler and Pedersen (1996). However in case of *C.setigerus* and *C.echinatus*, the highest callus growth though noticed on B_5 medium, was restricted to the first passage only, whereas, in subsequent passages of subcultures MS and SH media performed better for growth of the seed explant derived calli. Comparable callus growth on MS and B_5 media has earlier been reported by Wernick and Brettell (1982).

For seed explant, genotype EC400587 and for inflorescence explant, genotype EC400631 of *C.ciliaris* showed maximum callus multiplication. All the genotypes showed comparatively less callus growth rate in subsequent passages except EC400587 of *C.ciliaris* and *C.echinatus* in case of seed explant. Gupta *et al.* (2002) observed that once the callus had been induced its further growth depended on the inherent callus growth potential of the genotype. In case of the inflorescence explant derived calli, the growth rate in first passage was comparatively less than the second and third passages in general among all the genotypes which was contrary to the callus growth rate pattern observed in seed explant, showing comparatively less growth rate in the subsequent passages than the first passage. The initial lag in the callus growth rate from inflorescence explant might be due to amenability of comparatively very small fraction of inoculum tissue in the explant for callus induction and also that immature inflorescence produced more compact callus than the seed explant, hence, exhibited slower growth. Study on wheat showed that compact calli grew slower than the friable type of callus (Redway et al., 1990). The juvenility of the tissue in seed explant may also be ascribed for its higher callus induction potential than the inflorescence explant. Schenk and Hildebrant (1972) noticed less growth in certain monocotyledonous plant tissues

after culture for extended period. However, the response of two explants on a particular culture medium was comparable in sorghum (Gupta *et al.*, 2002).

The calli in general, exhibited a high potential for organogenesis or embryogenesis in first subcultures but this potential gradually declined with the proceeding subcultures and eventually lost their morphogenetic capability. Increase in number of subcultures leading to decreased regeneration potential was also noticed by Gao-Zhen *et al.* (1999).

(C) Regeneration

(i) Histological Observations

From the regeneration point of view, the compact white and nodular calli were found to be the most suitable. In the present study, the regenerating calli were composed of compact cell masses of comparatively uniform and smaller size with densly staining cytoplasm and prominent nucleus and intermingled with high starch granule containing cells. These regenerating cells were without intercellular spaces and exhibited meristematic activity. These types of cells were also noticed in regenerating of calli by Kohlenbach (1978) and Vasil and Vasil (1982). Vasil and Vasil (1981) observed that the embryogenic calli were characteristically compact, much organized and white to pale yellow in colour.

The most frequently noted regeneration response in the present study was the organogenic induction of shoot buds. These regenerated shoot buds were typically similar to the vegetative shoot apex in their histological organization and morphology bearing laterally subtending leaf primordial. Intermittent occurrence of somatic embryo development was also observed. The reports on *in vitro* regeneration in finger millet have also described regeneration through shoot bud formation (Rangan, 1976 and Mohanty *et al.*, 1985). Sivadas *et al.* (1990) reported that shoot bud formation was a major path way in finger millet. However most of the gramineceous crops showed regeneration through somatic embryogenesis as described in *Pennisetum americanum* (Vasil and Vasil, 1982), *P. purpureum* (Haydu and Vasil, 1981) and bahia grass (Shatters *et al.*, 1994). The somatic embryos exhibited the morphology of a typical monocot type of embryo showing bipolar organization, coleoptile and coleorhiza being

represented for shoot and root apices bearing scutellum laterally. The same type of morphology of somatic embryos has been reported in bahiya grass (Shatters *et al.*, 1994), *P. americanum* (Vasil and Vasil, 1982) and finger millet (*Sivadas et al.*, 1992). In addition to shoot bud organogenesis, formation of multiple shoot bud also occurred quite frequently among the regenerated mass in all *Cenchrus* genotypes. The organization of multiple shoot buds was also similar histologically and morphologically to that of vegetative shoot apex. Multiple shoot meristems have also been observed in the regenerating calli of *P.americanum* by Vasil and Vasil (1982), which developed into several plants.

(ii) Shoot differentiation

Organization could be brought about in calli by the controlled initiation of organ primordial through manipulation of the nutrient and hormonal constituents in the culture media. The role of cytokinin and auxin ratio in shoot differentiation was clearly observed. BA and kin with different levels were used as growth regulators for shoot induction. There was highly significant difference among different media combinations as well as among the genotypes in both seed and inflorescence explant derived calli used for regeneration. The best medium contained either 2 mg/l BA or 2 mg/l kin for all the three species. Addition of 0.2 mg/l 2,4-D to the cytokinin containing media was more effective than the media with cytokinin alone. In wheat, Fekete and Pauk (1989) also found significant increase in plant regenration by using 2,4-D at lower concentration (1-1.5 mg/l) together with kinetin in various combinations (0.5-4.0 mg/l). Davoyan (1991) showed highest regeneration frequency on a medium supplemented with 2,4-D (0.5 mg/l) and BA (5 mg/l). There was 14 per cent increase in regneration frequency with the increase in cytokinin concentration and lowering the levels of auxin when compared with media containing cytokinin alone (Srivastaqva and Chawla, 2001). However, some workers in their studies have observed that exogenous cytokinins are not required for regneration in all cases, probably because of adequate levels of cytokinins already present in some tissues (Norstog, 1970; Henke *et al.*, 1978 and Inoue *et al.*, 1979).

As observed in case of callus induction, there were noticeable genotypic differences for shoot formation also. Highest number of shoots/100 mg of callus was obtained in genotype EC400610 which was comparable to genotype IG693108 when the calli were raised from seed explant. Lowest performance was recorded in genotype EC400631. In case of immature inflorescence explant, genotype EC400631 was found to be the best in terms of number of regenerated shoots per 100 mg of callus. Genotypic differences were also observed in shoot differentiation and maintenance of regeneration capacity in sorghum (Cai and Butler, 1990 and Kosting *et al*,. 1996), in barley (Hanzel *et al.*, 1985; Rao *et al.*, 1992 and Dehleen 1999), in wheat (Rajyalakshmi *et al.* 1988 and Mathias, 1990), in corn (Green, 1977), in oats (Cummings *et al.*, 1976) and in sugarcane (Bhansali and Singh, 1982).

(iii)**Rhizogenesis**

Rhizogenesis among the *in vitro* regenerated shoots of most of the cereals (monocots) has been possible on a nutrient medium with or without an auxin, such as IBA and/or NAA (Bhaskaran and Smith, 1990). In the present experiment, different levels of IBA were used with MS medium for induction of rooting response in the *in vitro* regenerated shoots in order to develop plantlets. The best root induction response was recorded with 0.5 mg/l IBA + 2 g/l charcoal in all the genotypes of *Cenchrus* except *C.echinatus* and there was highly significant difference among media. Supplementation of charcoal showed enhancement in rooting capacity. IBA has also been observed to be quite effective for root induction in finger millet (Sivadas *et al.*, 1990) and sugarcane (Mohan Das, 1995). Other auxins, such as, NAA (Chin and Scott, 1977 and Varshney *et al.*, 1999), IAA (Diaz *et al.*, 1989) or lowering of cytokinin concentration (Bhaskaran and Smith, 1989) or increased sucrose levels (Diaz *et al.*, 1989) are also well known to induce luxuriant root formation. Bhansali and Singh (1982) obtained adventitious roots and callus in sugarcane on MS media with NAA or IAA. Rodriguez (1982) induced rooting in sugarcane by eliminating 2,4-D and subjecting the cultures to constant illumination. Application of charcoal in the medium has known to establish polarity by darkening the media, absorbing the inhibitor compounds present in

media and improving the aeration of the culture medium (Mohmed Yaseen *et al.*, 1995). NAA in *C.ciliaris* @ 0.1 mg/l induced root formation (Kacker and Shekhawat, 1991). Mohanty *et al.*, (1985) could induce roots on transferring the shoots to the MS media with half strength of mineral nutrients but devoid of growth regulators. In the present study also, the half strength MS medium showed response for root induction in all *Cenchrus* species, though moderately. Gentoypes of *C.ciliaris* did not show significant difference among them for rhizogenesis. However, the genotypes, IG693108 and EC397600 of *C.ciliaris* exhibited maximum number of roots formation.

(D) Survival of Plantlets

Habituation of the *in vitro* grown plantlets to the external harsh conditions has been observed as a gradual process. In the present study, plant survival was found maximum in *C.setigerus* followed by the genotype EC400631 of *C.ciliaris* while transferring them to pots from culture conditions. Rest of the other genotypes were intermediate for this response. In case of wheat also, the percentage of surviving regenerants and of the fertile plants was genotype dependent (Rakhimbaev and Kushnarenko, 1991). However in sugarcane, plant survival was almost 100% in hygroscopic system without any genotypic influence (Sukla *et al.*, 1994). Cummings *et al.* (1976) in oats, Junyan *et al.* (1988) in *Setaria* species and Kitvijarm (1985) in sugarcane, reported that the soil was the best potting medium. Plantlets of *C.ciliaris*, transferred to field after acclimatization in growth chamber at 26^0C, showed better survival (Kacker and Shekhawat, 1990).

From the foregoing discussion, it could be inferred that the culture medium in general, containing 2,4-D @ 3 mg/l or 5 mg/l with MS medium yielded good callus with appreciable regeneration potential. The callus could be further maintained on the same medium at least up to five subcultures and regeneration occurred satisfactorily on MS medium with 2 mg/l BA or 2 mg/l kin with 0.2 mg/l 2,4-D.

5.2 Somaclonal Variation

Somaclonal variations commonly appear in *in vitro* plants regenerated via callus phase. In present study, there has been an immense utility of somaclonal variation for inducing genetic variability due to apomictic mode of reproduction of this crop, which limits the genetic recombination. However, only heritable variations are of significance. In the present investigation somaclonal variations were evaluated on the basis of following studies:

(A) Morphological Observation

(i) Variations in Somaclones

Agronomically important characters were studied in all the regenerated plants. Bi-directional variations were observed for most of the quantitative traits in all the genotypes of all the three *Cenchrus* species (*C.ciliars, C.setigerus* and *C.echinatus*) which were studied for two seasons. The CV statistics allows direct comparison among different traits to determine those with the greater variability for each of the genotype and species. CV difference among the traits reflects the different response of various traits to the effects of adventitious regeneration.

With regard to genotypes of *C.ciliaris*, somaclones derived from the seed explants of the genotype EC400631 exhibited higher CV than the somaclones derived from immature inflorescence explant of the same genotype. In this genotype green fodder yield and dry matter yield were the most variable traits in somaclones. There was considerable difference in variation for different traits between the somaclones of different explant source. Explant sources varied in their ability to generate somaclonal variation (Skirvin *et al.*, 1994). Some differences in variation for agronomic traits were observed in rice plants regenerated from seed versus plumule culture (Heszkh *et al.*, 1989). Same results were also reported by Gui *et al.* (1993) and Chauhan and Sing (1995). Although the influence of tissue source would be most pronounced in poly-somatic species, yet D' Amato (1989) identified some plants, which did not fall into this category.

Mutations occur spontaneously and that changes observed in the somaclones might have arisen from mutant cell in the tissue source. Cells undergo cytological changes during differentiation resulting in the formation of mixploid tissues and organs in many species (D' Amato, 1985)

Qualitative or quantitative change in DNA during differentiation and development of a genotype (D' Amato, 1977) occurance of transposable elements (Nevers *et al.*, 1986) or presence of unsuitable genetic loci (Durrant, 1981) render a plant more susceptible to the production of variants from tissue cultures. Maximum variability was found in the somaclones of EC400631 for number of tillers and green fodder yield followed by the genotype IG693108 for internodal length. Somaclonal variation could also be influenced by the explant variety (cultivar) used. In oats (McCoy *et al.*, 1982), maize (Zehr *et al.*, 1987) and wheat (Mohmund and Nabors, 1990) reported the varietal differences in generating somaclonal variations.

Greater variation was found for most of the traits like GFY, DMY, number of tillers, Plant height, hundred bur weight and leaf width in *Cenchrus* species. In rice, variation in shooting ability, length and width of grain, peduncle length (Marrasi and Rapela, 1992), plant height (Oono, 1983 and Ming *et al.*, 1995), tiller number (Oono, 1983), grains/panical (Yang *et al.*, 1996) kernal length, early flowering, semi dwarf habbit and better grain quality and greater yield (Abbassi, 1999) have been reported. In maize Ro regenerants had a high growth rate and tillering capacity (Gasper *et al.*, 1995). The size of flag leaves, plant height, tiller/plant, spike length and seeds per spike differed significantly between SC_1 and the parent (control) in wheat (Hashim *et al.*, 1990). Contrasting colours of bur were noticed in some somaclones of the genotype EC400610 and in one somaclone of genotyope EC400587 as compared to the parental material. Variation in quantitative traits in mutation and tissue culture derived plants have been widely reported (Sun *et al.*, 1983). There have been several reports on the chlorophyll and other pigment related changes in SC_1 generation of many crop plants, such as, wheat (Eapen *et al.*, 1985), barley (Ahloowalia, 1987) and *Penisetum* species (Lauzan *et al.*, 1991).

(ii)Progeny analysis

In the present study, plants regenerated in *in vitro* cultures (SC_1) were allowed to self-pollinate and were threshed individually at maturity. In the next season, plant to row progenies (SC_2) were raised to study the variation. Heritable but recessive genetic changes could be detected generally by selfing of tissue culture regenerants and examination of the progeny for two or more selfed generations (Edallo *et al.*, 1981; Larkin *et al.*, 1984). Convincing evidence of heritable genetic changes has been obtained in only few instances. Many of the other reports of variability may be epigenetic, developmental or physiological in nature (Vasil, 1987).

In genotype IG693108 of *C.ciliaris* and *C.echinatus* very few progenies and their somaclones exhibited similar or near to similar values for a limited number of parameters. Irvin (1984) concluded that the occurance of permanent phenotypic changes is not a frequent phenomenon and that some of the observed changes may indeed be temporary or a result of chimerism.

Greater variation for GFY and DMY in the progenies of somaclones of genotype EC400631 for GFY and DMY and number of tillers in the genotype IG693108 and for GFY, DMY and number of spikelets per spike in the genotype EC400610 of *C.ciliaris* were recorded. In the progenies of somaclones of *C.setigerus* there was a great variation in GFY, DMY and number of tillers. The progenies of somaclones of *C.echinatus* showed variability for number tillers, internodal length, GFY and peduncle length.

Somaclonal variation among progenies of regenerants of numerous morphological and agronomic traits have been reported. Positive variations in height (Bhaskaran *et al.*, 1987; Ma *et al.*, 1987 and Cai *et al.*, 1990) Sterility (Ma *et al.*, 1987 and Elkonin *et al.*, 1994), maturity (Bhaskaran *et al.*, 1987), biomass (Bhaskaran *et al.*, 1987), grain yield (Sun *et al.*, 1983 and Mohmand and Nabors, 1990) tillering (Bhaskaran *et al.*, 1987) have been documented.

Somaclonal variation in tissue culture-regenerated material has been often, though rarely, traced to variability in the parental plant population (Breman *et al.*, 1989). This emphasizes the importance of designing of experiments which also account for the possibility of the variation arising from mutations already

existing in the source material which could be reflected by the analysis of donor plants amongst the control (Bebeli *et al.*, 1990) and by observations that the same mutation was not present in other plants regenerated from the same culture (Brettellet *et al.*, 1986)

The study of the second generation of the regenerated plants would help to differentiate between heritable and non-heritable variations. In the present study, plants were showing large deviation from parent plants in both the directions for many agronomic traits. GFY and DMY were the more variable characters in progenies of most of the somaclone of all genotypes except *C.echinatus.*

Stability for different characters in diverse genotypes of *Cenchrus* showed the inheritance of variation induced in somaclones. On the basis of frequency distribution. Progenies of somaclone of the genotype EC400631 and EC400631 of *C.ciliaris* and *C.setigerus* exhibited stability for various agronomic traits like plant height, number of tillers, leaf width, internodal length, spike length, GFY, DMY, spike width, and bur colour. However, the genotype, IG693108 of *C.ciliaris* and *C.echinatus* showed variation in preogenies for most of the characters.

In rice, Marrasi and Rapela (1992) found inheritance of some characters, *viz.*, shooting ability, husk colour, length and width of grain, panicle length upto the second generation of somaclones. Chauhan and Singh (1995) tested somaclones for kernal bunt resistance in R_0 generation. Resistance was inherited upto R_3 generations. Li *et al*, (1995) found heritable and stable variation in progenies of plant regenerated from cultured immature embryos of wheat.

Somaclones of *Paspalum dilatum* expressed drought and heat tolerance and they could be permanently fixed in subsequent generations due to its apomictic nature (Tischler *et al.*, 1993).

Bi-directional changes were found in all the genotypes of *C.ciliaris, C.setigerus* and *C.ehinatus* revealing that somaclonal variations might be both positive or negative. Negative variation was also observed by Qureshi *et al.* (1992) in wheat. They also observed that in spring wheat, positive variations

were associated with some negative alterations. Similarly, Baillie *et al.* (1992) observed that a field evaluation of somaclones of barley had little variation and that was of negative value.

A synergistic and concerted effort by traditional breeders and plant biotechnologists could bring this technology (Somaclonal variation) to potentially efficient level for its application that would result in cultivars with improved value-added traits (Rowland *et al.*, 1995).

(B) Isozyme analysis

(i) Isozyme pattern

Isozyme studies have been especially useful in tissue culture analysis. Enzymes like EST, SOD, PGM, PGI, G6PDH and ACP have been used for the analysis of tissue culture derived progeny. In the present investigation, scrutiny of a larger number of enzymes has been done than the most other studies, including those that detected variation. Eleven progenies of genotype EC400631, two progenies of genotype EC397600, three progenies of IG693108, one progeny of EC400587, three progenies of EC40061, two progenies of EC397680 from *C.ciliaris* and one progeny of EC400639 from *C.setigerus* including the parent of all the accessions were characterized in the present study using 6 enzyme systems. Qualitative differences were observed between progeny with their respective parents indicating somaclonal variation. Only SOD in EC400631, EC400587 and IG693108 could not differentiate the progeny from their respective parents indicating its least polymorphic nature. Whereas, EST, PGM and PGI enzymes exhibited highest polymorphism across all the parents-progenies. Thus, five of the six enzymes showed explicit qualitative differences between parents and their progenies indicating the occurrence of somaclonal variation.

Maximum (10) electrophoretic phenotypes (EP) were observed for EC400610 followed by EC400631 (8). In case of EC400631, electrophoretic phenotypes of two progenies from seed explant for PGI were the same, whereas other nine progenies had shared seven different Eps which indicated that PGI isozymes could be effectively used for distinguishing tissue culture derived progenies

from different explant sources. Similar observation was recorded for progenies of EC400610. This nature of polymorphism of PGI was used in many other studies. Humphrey and Dalton (1992) observed a direct relationship in regenerated plants between time in cell suspension and the number of aberrations at the PGI/2 locus. The stability of *Lolium* and *Festuca* PGI/2 homeoalleles in cell suspension culture was described and was related to the cytological data. In another study to identify and charaterize genetically novel mutations in soybean regenerants, variant isozyme patterns were observed in two independent tissue culture derived lines. Genetic analysis were conducted on these two isozyme variants and they were heritable. The frequency of soyabean somaclonal isozyme mutants in that study was two out of 185 R_0 plants.

The present study has shown high frequency of variants among parent and their tissue cultured derived progenies which could be due to the polyploid nature of buffel grass in general and the extent of heterozygosity in specific. This is the first report of isozymic variants among tissue culture derived progenies of buffel grass. On the contrary, biochemical analysis of the plants derived from embryogenic tissue cultures of napier grass, a related species to buffel grass, has shown no qualitative variation at any of the loci examined out of 14 isozyme patterns (Shenoy and Vasil, 1992). Their study indicated genetic uniformity of plants derived from somatic embryos.

One of the major objectives of the present study was to develop somaclonal variation in buffel grass that could be exploited in the breeding programmes. Owing to its apomictic nature, genetic improvement of *Cenchrus* is limited to selection methodologies. Hence somaclonal variants for novel traits were desired for breeding. Biochemical analysis of tissue culture derived progenies in one of the widely used method to ascertain somaclonal variation (Larkin and Scowcroft, 1981). Since enzymes are coded for by the genes and any disruption in the coding sequence even at the single base level could force variation in the expression of the enzyme, leading to an altered individual. Any aberrations in the DNA structure that leads to an altered expression in enzymes belonging to

major biochemical pathways would most likely be lethal and hence not expressed.

During our study, great variation was observed for the genotype EC400631 in terms of number of spikelets/spike, panicle length and internodal length. These somaclones also exhibited isozymes variation in EST, PGH, PGI and ACP systems. Another genotype EC400587 and some of its somaclones showed green bur colour where as the seed derived progenies (R_1) had purple bur colour. Such mutations were substantiated by Evan and Sharp (1983) and Lee and Phillips (1988) who had observed changes in tissue culture derived progenies due to change in chromosomal number or their rearrangements. Most importantly, during this study in *C.setigerus*, the somaclone was reproducing through facultative apomictic mode while its source material had reproduced apomictically. Similar observations were made by Yamagishi *et al.* (1997) who observed varied quantitative traits such as heading date, number of spikelets per panicle and seed fertility, which were due to their heterozygous state.

The high amount of variation in the somaclone of *Cenchrus* could also be attributed to the heterozygous nature of protoclones especially for quantitative traits and reproduction. The same was corroborated by Dahleen and Eizenga (1990) who logically concluded that the variation in isozyme patterns was due to a mutation or deletion, since they had a somaclone with a loss of exptression of an allele.

(ii) Dendrogram analysis

Occurrence of six clustures in the dendrogram and the grouping of somaclones of each parent in a single cluster clearly indicated the genetic relatedness of somaclones to their respective parents. This distribution also indicated the usefulness of dendrogram analysis in understanding the phylogenetic relationship among parents and their tissue culture derived progenies. Fifth cluster appeared as most heterogeneous with three genotypes and their few somaclones together. This distribution further strengthened the observation that somaclones generated from this study were indeed genetically heterogeneous with their obvious usefulness as breeding materials. This heterogeneity could be easily ascribed to the heterozygous nature of the protoclones used in the

study. Another interpretation for such heterogeneous grouping in 5th cluster could be the type of material chosen for tissue culture. They seemed to be genetically closer in comparision to other protoclones. Except this cluster, rest of the clusters have almost shown each protoclone with its derived somaclones. This study will be useful for selecting parents and somaclones as per their genetic distance to maintain variation among tissue culture derived material during breeding.

(C) Chemical analysis

Genotype EC400631 (100000) had lower CP (1.84) and more cell wall contents accumulation than its somaclones (3.05) and progenies (3.38%). Further the AI value of parent was 5-7 units lower than its somaclones and progenies. Genotype EC397600 (200000) revealed that parent had higher CP and fiber contents compared to somaclones and progenies. Parent had 9 units lower AI values than its progenies. In genotype EC400587 (30000) and parent had lower CP higher higher cell contents (NDF, ADF, cellulose and lignin) than its somaclones and their progenies. In genotypes EC400631, EC400587 EC397680 of *C.ciliaris* and *C.setigerus* (EC400639) parent had lower CP and higher fiber fractions (NDF, ADF, cellulose and lignin) as compared to its progenies and somaclones, while in genotypes EC397600 and EC400610, parents had less CP content bud had more accumulation of fiber fraction. Availability index values of parents and their somaclones and progenies varied between 62.9-83.7 and 67.44-78.91 and 64.42-86.57 per cent, respectively. The variability in CP content and cell wall constituents (NDF, ADF, cellulose and lignin) amongst parents somaclones and progenies of evaluated genotypes of *C.ciliaris* are within the range of reported values (Pandey *et al.*, 1977; Singh and Samanta, 1998, Pachauri *et al.*, 1998 and Singh, 2001). However, higher CP values have been reported by other workers (Dass *et al.*, 1978 and Kanodia and Parihar, 1988). This variability in chemical entities of the parents, somaclones and progenies of tested genotypes may be attributed to several factors *viz.*, stage of the plant growth, environment, fetilization and genetic make of plant. AI values of different genotypes varied due to the differences in their cell contents (NDS) and lignin concentration.

SUMMARY

SUMMARY

The results of various experiments conducted in the present study have been categorized under two major groups. The first major group has been comprised of the experiments on optimization of induction, quality and growth of the callus and regeneration of plantlets from different explants in six genotypes EC400631, EC397600, EC400587, IG693108, EC400610 and EC397680 of *C.ciliaris*, *C.setigerus* (genotype EC400639) and *C.echinatus* (genotype EC397342). The second major group consisted of experiments on evaluation of somaclones and their progenies in comparison with the respective parent plant materials of the above mentioned genotypes for various morphological traits, isozymes and chemical analysis.

For callus induction frequency, the effect of different basal media (B_5, MS and SH) from seed and inflorescence explants revealed that MS medium performed better than the other two media in all the genotypes of the three *Cenchrus* species. *C.echinatus* showed best performance in terms of callus induction frequency with the seed explant while immature inflorescence explant gave best response in *C.setigerus*. Seed explant of IG693108 and inflorescence explant of EC400587 performed best for callus induction in *C.ciliaris*.

All the three *Cenchrus* species performed quite well for callus quality (colour and texture) on MS medium. The genotypes EC397600 and EC400631 of *C.ciliaris* exhibited the best quality of callus with seed and inflorescence explants, respectively.

The effect of different levels of 2,4-D in the media for callus induction frequency from seed explant revealed that the application of 5.0 mg/l 2,4-D was most suitable in all the three *Cenchrus* species. However, inflorescence explant of *C.ciliaris* and *C.setigerus* gave best results on the media containing 3.0 mg/l and 2.0 mg/l 2,4-D, respectively. The genotypes, EC400610 and EC400631, both of *C.ciliaris* showed maximum callus induction frequency with seed and immature inflorescence explant, respectively.

With regard to callus quality (colour and texture), 2,4-D @ 3.0 mg/l was found to be optimum for both the explants in *C.ciliaris* and *C.setigerus*. However,

C.echinatus did not how any difference in callus quality with the application of different levels of 2,4-D. Better quality of callus, on the basis of average of scores for colour and texture, was exhibited in the genotypes EC397600 and EC400631 of *C.ciliaris* for seed and inflorescence explants, respectively.

Effect of different levels of BA applied in the cultures for studying callus induction frequency showed that the maximum callus induction from seed explant of *C.ciliaris* and immature inflorescence explant of *C.setigerus* were obtained on the medium containing 0.5 mg/l BA. However, immature inflorescence explant of *C.ciliaris* and seed explant of *C.echinatus* performed best on the medium containing 0.5 mg/l BA + 500mg/l CH, whereas the seed explant of *C.setigerus* performed best in the medium without any application of BA.

For callus quality, seed explant of EC397680 and immature inflorescence explant of EC400631 genotypes of *C.ciliaris* elicited best callus types on the medium containing 0.5 mg/l BA + 500 mg/l CH. Both the explants of *C.setigerus* also performed at par on this medium however, no much difference in callus quality was observed with different levels of BA in *C.echinatus*.

Callus growth studies were carried out by subculturing the calli at a regular interval of 20-21 days. The callus from seed explant of genotypes EC400587 of *C.ciliaris* showed maximum multiplication rate on MS medium. However, the growth rate, in general, declined in subsequent subcultures. MS medium elicited maximum callus growth rate from immature inflorescence explant also and the genotype EC400631 of *C.ciliaris* performed best. However, callus growth rate in the first passage was less than the second and third passage which was contrary to that of the callus growth in seed explant.

The regeneration studies were carried out with the best quality of calli obtained from both of the explants of different genotypes. The regenerating calli were composed of the compact mass of cells of uniform and comparatively smaller size with densely staining cytoplasm and prominent nuclei. The regeneration was most frequently observed through shoot bud organogenesis with occassional occurrence of somatic embryogenesis. The differentiating shoots exhibited monopolar structures of typical apical shoot bud morphology and tunica corpus organization. In addition to shoot bud regeneration, multiple shoot bud formation was also

observed which showed similar type of morphology. The somatic embryos showed bipolar organization with the morphology of a typical graminaceous embryo.

All the genotypes of *C.ciliaris* and *C.setigerus* showed maximum shoot induction frequency from the calli of seed and inflorescence explants, both on the media containing 2.0 mg/l BA or kin along with the application of 0.2 mg/l 2,4-D. However, seed explant of *C.echinatus* performed better on the medium containing 2.0 mg/l kin alone. Best regeneration performance was observed from the calli of genotypes, EC400610 and EC400631 of *C.ciliaris* for seed and immature inflorescence explants, respectively. In general, the application of 0.2 mg/l 2,4-D in the medium together with cytokinin proved beneficial for regeneration of shoot buds.

The regenerated shoots were placed on rooting media for rhizogenesis. The maximum rooting response was observed in the genotype, EC400587 of *C.ciliaris*. Highest number of roots were counted on the media containing 0.5 mg/l IBA + 2 g/l charcoal in case of *C.ciliaris* and *C.setigerus*. *C.echinatus* showed best rooting efficiency on media containing 1.0 mg/l IBA + 2g/l charcoal. Addition of charcoal enhanced the rooting response.

The plantlets with well developed shoots and roots were subjected to hardening for their acclimatization from cultures to field conditions. Genotypic difference for hardening also was observed.. Among all the genotypes of *C.ciliaris*, EC400631 showed highest survival rate (63.6%). In *C.setigerus*, 85 per cent and in *C.echinatus* 58 per cent survival was observed during the transfer of plants from pots to the field.

Regenerated plants, the **somaclones** and their progenies were raised in the field and were evaluated for variation as against their respective parents for various parameters.

Somaclones in different genotypes of *C.ciliaris* showed wide range of morphological variations from their respective parent. Uni and bi-directional variations were recorded in both the seed and inflorescence derived somaclones. The variation in green fodder yield (GFY) and dry matter yield (DMY) were the most common to all the genotypes of *C.ciliaris*. In addition to GFY and DMY, the genotype EC400631 exhibited variation for number of spikelets per spike,

peduncle length and Internodal length, the genotype, EC397600 showed variation for hundred bur weight, number of leaves per tiller, leaf width and leaf length, the genotype IG693108 exhibited variation in number of tillers and number of spikelets per spike, the genotype, EC400610 exhibited variation for number of tillers and internodal length and in the genotype, EC397680, number of tillers, spike width, peduncle length, leaf width and plant height were the more deviating characters.

In *C.setigerus* variations observed in the somaclones were more pronounced for GFY, DMY and number of tillers.

In *C.echinatus*, greater variability was recorded for GFY, leaf width, internodal length, spike width and number of tillers among the somaclones as compared to their parent.

The variations among the somaclones of *C.ciliaris* and *C.setigerus* recorded for two subsequent years showed similar trend, though with slight deviation. However, GFY recorded in second year showed marked increase in the somaclones. *C.echinatus* being an annual species, the data of only one year could be recorded and compared.

Frequency distribution of somaclones and progenies showed the similar or dissimilar pattern of distribution in various frequency classes. This exhibited stabilization of variation for various parameters in the progenies of respective somaclones. A large number of progenies of most of the somaclones among all the three species of *Cenchrus* showed stabilization for leaf length, DMY, spike length and hundred-bur weight. Different genotypes of *C.ciliaris*, *C.setigerus*, their somaclones and the progenies exhibited semi-erect type of growth habit. However, a slight deviation towards prostrate or erect types growth habits in the somaclones and more particularly in their progenies was observed in subcequent year. In case of *C.echinatus*, the parent, most of the somaclones and their progenies exhibited prostrate growth habit.

Leaves with smooth surface were found in most of the somaclones, their progenies and their respective parents among all the three species of *Cenchrus*. With regard to bur colour, variation was found in the genotypes EC400587 and EC400610 of *C.ciliaris*.

Somaclones exhibited variation in most of the isozyme studied. Zymogram of all the somaclones of *C.ciliaris* and *C.setigerus* mostly differed from that of their respective parents for EST, PGM and PGI isozymes. This clearly indicated presence of somaclonal variation. Number of electrophoretic phenotypes ranged from 1-8. The extent of somaclones differing from their respective parents varied from 0-100 percent.

Dendrogram analysis of the somaclones and their respective parents exhibited six broad clusters. Fifth cluster consisted of maximum number of genotypes. EC400631 was genetically the most distant genotype from EC397600. *C.setigerus* was closer to the genotype EC397600 of *C.ciliaris* and placed in sixth cluster. Dendrogram showed that parents and its somaclone were genetically similar. However, the heterogenous distribution of various somaclones generated from this study could be used to select genetically diverse somaclones for further improvement.

In chemical analysis, bi-directional variations were observed for most of the fodder quality traits also in various somaclones and their progenies in most of the genotypes of *C.ciliaris* and *C.setigerus*. Crude protein was found more in the somaclones and their progenies of genotypes EC400631, EC397680 and EC400610 of *C.ciliaris* and *C.setigerus* (EC400639). NDF was found more in the somaclones of genotype IG693108, EC400610 and EC397680 of *C.ciliaris* and *C.setigerus* (EC400639) while ADF content was higher in the somaclones of EC400587 of *C.ciliaris*. AI value was observed higher in the somaclones and progenies of EC400631 and EC397600 of *C.ciliaris*.

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